SCIENTIFIC CRITERIA DOCUMENT FOR MULTIMEDIA STANDARDS DEVELOPMENT POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

PART 1: Hazard Identification and Dose-Response Assessment

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FOR MULTIMEDIA STANDARDS DEVELOPMENT

POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

PART 1: Hazard Identification and Dose-Response Assessment

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1 EXECUTIVE SUMMARY

1.1 Probability and Risk

Section 1.1 provides a short introduction to the concept of probability and risk. Readers who are familiar with these concepts can proceed to section 1.2.

In the present report, we often discuss *risk*. Technically, risk is defined as the probability of an adverse event happening and can be expressed as the number of occurrence per hundred thousand or per million events. The risk of breaking a bone while performing a given task, for example, is 3 in a million. This statement means that if one million individuals partake in this activity, *on average*, three persons would break a bone. It does *not* suggest that, out of a million people performing the task, exactly three individuals would break a bone. The result is similar to tossing a coin. If we were to toss a coin 10 times, on average, tails would turn up 5 times. Few would be surprised, however, if four or seven tails appear out of ten tosses. On the other hand, ten tails would not be expected in ten tosses. Therefore, a risk of three in a million means that out of a million people performing a dangerous task, zero, two, five or even ten person(s) may actually break a bone. The probability of breaking a bone applies to large groups of people but not to individuals. Thus, if an individual is a smoker and develops lung cancer, one cannot prove that the individual has developed cancer *because* of his/her smoking history based solely on the high risk of lung cancer from smoking .

Rather than expressing risk as the number of cases in a million, risk is usually expressed in scientific notation. Thus, $5.6 * 10^{-6}$ (or 5.6 E-6) would mean 5.6 cases per million people exposed, while $3 * 10^{-3}$ (or 3 E-3) would mean three in a thousand people.

Low probabilities are often quite difficult to visualize in concrete terms. We find the analogy with playing cards useful for ourselves and we hope you will too. Imagine you are dealt a hand of thirteen cards out of a deck of 52 cards, your probability of getting a certain number of spades is finite (summarized in Table 1.1.1). Table 1.1.1 is provided merely to help you to have some feel for low probabilities. In the present document, however, we are dealing with serious human health risk and the probabilities associated with cards and adverse health effects are not comparable.

Readers who wish to know more about the levels of risk in our every day lives may enjoy reading "The Book of Risks" by L. Laudan (published by John Wiley & Sons, Inc.).

Table 1.1.1 Probability of being dealt spades in a hand of thirteen cards (from a 52 card deck).

Spades/deal	Chance of occurrence	
5 spades	1.3×10^{-1}	close to 1 in 10
7 spades	8.8×10^{-3}	close to 1 in 100
8 spades	1.2×10^{-3}	close to 1 in 1000
9 spades	9.3×10^{-5}	close to 1 in 10,000
10 spades	6.1×10^{-6}	over 1 in a million
13 spades	1.6×10^{-12}	close to 1 in a trillion

1.2 Introduction

Polycyclic aromatic hydrocarbons (PAH) have a long and notorious history as human carcinogens. Their discovery as carcinogens is tied to the history of coal tar. In 1775, Sir Percivall Pott reported an increased incidence of scrotal cancer among English chimney sweepers. Dr. Pott assumed that the cause of cancer was the soot and tar the sweepers came into contact with while cleaning the chimneys[1]. But it was until 1930 that PAH were first identified as the cause of coal tar carcinogenicity.

Since 1930, scientists have discovered that incomplete combustion of organic material always generates a large number of PAH. They are found in complex mixtures, namely automobile exhaust fumes, cigarette smoke, barbecue smoke, emissions of coking operations in steel mills, and many other emissions. PAH are also found in asphalt and tar, and are even added as tar to some medications

PAH are not generated singly, but rather as a component of complex mixtures which are made up of hundreds of PAH and other compounds. Many of the complex mixtures have been shown to be carcinogenic in a variety of animal species and some in humans. Although the toxicity of a vast majority of individual PAH is at present not fully known, much evidence indicates that some of the PAH (but not all) present in the complex mixtures are carcinogenic in many animal species. Despite the lack of direct evidence, the same PAH are generally assumed to be carcinogenic in humans.

Other than carcinogenic effects, PAH exhibit a wide range of toxic effects, including skin and eye irritation, immunotoxicity and developmental toxicity. Although many of the effects, including carcinogenicity, have been demonstrated at high experimental doses, carcinogenic effects are believed

1 Bjørseth and Becher, 1986

to occur at lower doses than other toxic effects. As a result, carcinogenicity is by far the best studied toxic effect of PAH and the effect on which all previous PAH risk assessments have concentrated their effort.

For the purpose of the present report, PAH will be defined chemically as compounds with nuclei of three or more benzene rings, where the neighboring rings share two carbon atoms. This definition is very open-ended and a large number of compounds with extremely diverse structures belong to this group.

PAH have been found at different concentrations in all media, including soil, air, vegetation, sediments, surface and drinking water, and food. Unfortunately, most of the environmental monitoring efforts in the past focused on only about a dozen PAH. As a result, the environmental levels of hundreds of other PAH largely remain unknown.

PAH are recognized to be one of the most important group of environmental pollutants, not only because of the potency of some PAH and the potency of the complex mixtures which contain them, but also because of the numerous natural and man-made sources which contribute to their ubiquitous presence in the environment. As such, PAH are on a number of lists of priority pollutants (e.g. the MOEE Candidate Substances for Bans, Phase-Outs or Reductions prepared by Socha *et al.*[2], the Floor List of OSHA Hazardous Chemicals that appears in the Chemical Guide to SARA Title III[3], the Toxic Waste, Hazardous Constituents, and the Groundwater Monitoring List under the US Resource Conservation and Recovery Act (RCRA), the ATSDR[4] list of Hazardous Substances prepared by the USEPA and ATSDR for the Superfund program, Hazardous Air Pollutants under the US Clean Air Act - see Book of Lists for Regulated Hazardous Substances published by Government Institutes, Inc. in 1992)

Despite their internationally recognized importance and the vast volumes of scientific literature[5] written on these toxicants, and the fact that PAH are among the oldest known environmental pollutants, the regulation of PAH in most jurisdictions lags far behind other environmental toxicants. The main reason lies in the enormous difficulty of quantitatively evaluating PAH toxicity. Some of the difficulties have been discussed in earlier text. Briefly, the environmental levels of a large majority of PAH and their toxicity remain largely unknown; the interaction among individual PAH as well as the interaction between PAH and other compounds in the mixtures are poorly understood; human toxicity data are

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2 Socha, A.C., Aucoin, R., Dickie, T., Angelow, R.V., Kaus, P. (1993) Candidate Substances for Bans, Phase-Outs or Reductions - multimedia revision, Ontario Ministry of Environment and Energy.

3 Clansky, K.B. ed. (1991). Chemical Guide to SARA Title III. Fourth edition. Ryotech Publications, Inc.

4 Agency for Toxic Substances and Disease Registry

5 Ontario Ministry of Environment and Energy sponsored a literature search which identified almost 26 thousand papers published on PAH between January 1982 and early 1986. We do not know the total number of papers published since 1930.

available for only a handful of complex mixtures that contain PAH. In addition, most of the experimental data available consider the toxicity of PAH painted onto the skin of mice, while it is generally believed that most of the human toxicity of PAH results from inhalation or ingestion.

Taking into account the ubiquitous presence of PAH and the toxicity of these compounds, the Ontario Ministry of Environment and Energy has decided to develop multimedia standards for PAH aiming at protecting human health. This Scientific Criteria Document (SCD) is the first step in that process. It is expected that the eventual standard that protects human life will also safeguard non-human terrestrial biota. Parallel efforts are currently under way to develop standards to protect aquatic biota.

In general, risk assessment consists of the following main components.

- **Hazard Identification**

  Hazard identification is the descriptive component of the risk assessment process and discusses the various toxic effects of PAH.

- **Dose-response Assessment**

  Dose-response assessment estimates the potency of individual PAH and the potency of the PAH fraction of complex mixtures.

- **Exposure Assessment**

  Exposure assessment estimates the levels of PAH to which the people of Ontario are exposed. We consider the oral, inhalation, and dermal routes separately and also account for the differences in the geographical location and the lifestyle of Ontarians.

- **Risk Characterization**

  Risk characterization estimates the risk of PAH exposure for Ontarians in different geographical locations and according to their lifestyles.

The present report covers the **Hazard Identification** and **Dose-response Assessment** components of the risk assessment exercise. In addition, the report highlights conclusions that are based on strong scientific evidence and conclusions that are based on professional judgement. Recommendations are summarized in section 1.6 and discussed in section 3.10.

1.3 Hazard Identification

1.3.1 Physicochemical Properties

PAH are organic molecules consisting of at least three rings, at least two of which are fused benzene rings. Any two neighboring rings share two adjacent carbon atoms. In some PAH, one carbon atom is substituted by an atom of another element, such as nitrogen, oxygen, sulphur, or halogen.

PAH are generally insoluble in water but can be readily solubilized in organic solvents or organic acids. This means that in aqueous environments, PAH are generally found adsorbed on particulates and on humic matter, or solubilized in any oily contaminant that may be present in water, sediment and soil. The solubility of PAH in water is inversely proportional to the number of rings it contains. Thus, three-ring PAH tend to be more soluble in water than the five-ring compounds.

PAH are solids at room temperature. Since PAH tend to have low vapor pressure, they are usually adsorbed on particulate matter in the atmosphere. The vapor pressure of a PAH is inversely proportional to the number of rings it contains. Thus, almost all five-ring compounds are particulate-bound, while three-ring PAH are also present as vapor in the atmosphere.

In the presence of sunlight, PAH undergo photooxidation in the atmosphere. For example, about half of a given quantity of benzo[a]pyrene (B[a]P) will be oxidized in a matter of hours to days. Photooxidation occurs much faster for particle-free PAH than particle-bound compounds. PAH in the air can also be oxidized by ozone, by reactive compounds adsorbed on the particles, by NO_x and by SO_x.

1.3.2 Toxicokinetics

Section 1.3.2 summarizes the information on the absorption, metabolism and elimination of PAH in humans.

Ontarians come in contact with PAH primarily through ingestion and inhalation. Under normal circumstances, dermal contact with PAH is relatively unimportant. Similar to other oil-soluble compounds, PAH are generally well absorbed, but are stored only briefly in the body, primarily in the kidney, liver and spleen. Most of the absorbed dose is then excreted into the bile, and eventually into the faeces, and to a much lesser extent, the urine. Most of the PAH are excreted in their metabolized forms and only very small amounts of the parent compounds find their way into the faeces and urine.

PAH are highly soluble in fats. They can rapidly enter cells and become virtually unavailable for excretion. Metabolic processes tend to make PAH more water soluble, thereby facilitating excretion. A number of metabolic processes compete to produce a variety of different metabolites. The competing processes include *phase I* reactions, which add one or more hydroxyl groups to the parent core, and *phase II* reactions, which attach highly hydrophilic groups to the PAH molecule. The phase I reactions are catalyzed by epoxide hydrolase and by a subset of cytochrome P-450 mixed-function oxidases known as aryl hydrocarbon hydroxylase (AHH).

Although the structures of PAH vary greatly, the compounds have similar metabolic fate, thus leading to the formation of homologous metabolites. The difference in the tumorigenicity of different PAH is due to the difference in the location where metabolic modification takes place and the difference in the reactivities of intermediate metabolites. Some metabolites formed are diol epoxides,

some of which are in turn converted into carbonium ions. The carbonium ions can react with DNA and proteins to form adducts, and induce genotoxic damage. It is these alkylating agents that are thought to be the primary carcinogens, acting as initiators. Initiation is the first step in the development of cancer.

The enzymes required for the conversion of parent PAH compounds into the reactive diol epoxides are found mainly in the liver. These enzymes are also present in the lungs, the skin basal cell layer, the intestinal mucosa and other tissues.

1.3.3 Toxicity

PAH have been shown to induce a number of toxic effects. Some of them, however, are unlikely to be a cause for concern at environmental levels for Ontarians. Several PAH have been shown to cause death in rodents after short-term exposure to high doses. On the other hand, no deaths have been reported from short-term occupational exposures in humans. Since environmental levels are generally much lower than occupational exposures, it is extremely unlikely that short-term exposure to PAH in the environment would cause death. On the other hand, eye irritation, photophobia, and skin toxicity such as dermatitis and keratosis, have been demonstrated in workers occupationally exposed to PAH. We expect that extreme environmental conditions (e.g. heavy exposure to a forest fire smoke) may also trigger the same effects.

Adverse respiratory effects, including acute and subacute inflammation, and fibrosis, have been demonstrated experimentally. With B[a]P, severe and long-lasting hyperplasia and metaplasia were observed. These effects manifest themselves as precancerous lesions and are consistent with the general assertion that one of the main targets of PAH toxicity is the respiratory tract. Available data are insufficient to assess the effects of PAH at environmentally relevant concentrations.

Carcinogenic PAH, but not the noncarcinogenic ones, have been reported to suppress the immune reaction in rodents. A number of authors have reported immunosuppressive effects at a similar dose-range at which carcinogenicity has been observed. Furthermore, the potency of PAH as immunosuppressants appears to roughly correlate with the potency of PAH as carcinogens. Besides being an important endpoint, immunosuppression may be involved in the mechanism by which PAH induce their toxic effects including cancer. However, the data on immunosuppression are at present not sufficient for quantitative assessment.

Exposure to PAH can have adverse effects on both female and male reproductive systems and on fetal development. Most of the available data relate to rodent fetal development. Adverse effects include malformations, stillbirths, resorptions, immunosuppression, clastogenicity, and tumorigenicity. The doses required to produce the reported effects are generally similar or somewhat

higher than those required to elicit a carcinogenic response. Although no human data are available, reproductive and developmental effects may be important in humans. Unfortunately, available data are insufficient to quantitatively assess both reproductive and developmental effects.

Genotoxic effects have been repeatedly demonstrated for some PAH, both in *in vivo* tests in rodents, *in vitro* tests using mammalian (including human) cell lines, as well as in prokaryotes. On the other hand, some PAH appear not to be genotoxic. Most of the unsubstituted PAH which are categorized as genotoxic are not genotoxic in themselves, but need to be metabolized first by the AHH system. The diol epoxides formed then react with DNA to form DNA adducts and thus induce genotoxic damage. A genotoxic event is postulated as a required step in the carcinogenic process and may play a role in some forms of developmental toxicity. In our assessment, we do not consider genotoxicity as a toxic endpoint by itself, but rather as a necessary step towards carcinogenicity and some forms of developmental toxicity. For this reason, we do not use genotoxicity data directly in our dose-response assessment.

The tumorigenicity and carcinogenicity of individual PAH and PAH-containing mixtures have been well studied in experimental animals. A number of individual PAH are carcinogenic, while others have been found to be non-carcinogenic. For the rest of the PAH, the available data are insufficient to determine whether they are carcinogenic or not. In humans, virtually no data exist regarding the carcinogenicity of individual PAH, and only a limited amount of data is available for PAH-containing mixtures. However, the available evidence demonstrates unequivocally that some PAH-containing complex mixtures are carcinogenic both to humans and experimental animals. The site and the type of tumors are dependent on both the species and the route of administration.

1.3.4 Conclusions

Section 1.3 has summarized the numerous toxic effects ascribed to PAH. We expect effects such as skin, eye and respiratory mucosa irritation more likely to be associated with high occupational exposures rather than with the characteristically lower environmental exposures. There may be exceptions. Exposure to heavily contaminated soils or sediments, for example, may trigger these effects. Nevertheless, these kinds of effects have not been studied well enough to allow for quantitative assessment.

PAH-induced immunotoxicity and developmental toxicity may be important toxic endpoints. Unlike carcinogenicity, there is a paucity of human data for immunotoxicity and developmental toxicity. Furthermore, animal data in support of the carcinogenicity endpoint are far more extensive than for immunotoxicity and developmental toxicity. Assuming that the tumor-initiating effect of PAH has no threshold (a generally accepted assumption), these toxicants are expected to pose a finite cancer risk even at extremely low doses. In contrast, it is not clear whether a threshold exists for PAH immunotoxicity and developmental toxicity. It is plausible that, at environmentally rele-

vant(low) levels, PAH would not exhibit appreciable immunotoxicity and developmental toxicity. We have therefore chosen to conduct a dose-response assessment for PAH based on their tumorigenicity/carcinogenicity effects.

1.4 Dose-Response Assessment

1.4.1 Background and Rationale

The purpose of the dose-response assessment is to estimate the relationship between the risk and the dose of PAH responsible for the risk. Our goal is not to assess the risk due to individual PAH per se, but rather the risk due to the PAH fraction of the mixture. Our task is considerably more complex than what is involved in evaluating the risk of a specific toxicant. Environmental mixtures typically consist of hundreds of PAH and many other compounds. Some PAH are among the most carcinogenic compounds known to humans, while others appear to be noncarcinogenic. Unfortunately, the toxicity of the vast majority of PAH is unknown. Among PAH whose toxicity is known, many are not found in the environment and are synthesized only in the laboratory. Others are found in the environment but they are not routinely monitored for. In fact, among the hundreds of PAH reported to exist in the environment, only a handful are routinely monitored. As a result, only approximately a dozen PAH have both good toxicological **and** analytical data. We consider it unlikely that this small group of compounds is representative of the hundreds of other PAH found in a typical complex mixture and of their toxic properties.

An additional problem arises as a result of the possible interaction among PAH in the mixture as well as the interaction of PAH with many other (non-PAH) compounds found in the complex mixtures. While there seems to be a general consensus in the scientific community that PAH effects are approximately additive or somewhat less than additive, the mixtures may contain compounds that act through different mechanisms, and interaction between such compounds is often not additive, but synergistic (more than additive).

We are therefore faced with a challenge. We can approach the problem by estimating the potency of individual PAH for which data are available and summing up the risks attributable to each of these compounds. This approach has been used by many authors, particularly in North America. We are reluctant to depend on this approach, however, because we suspect that the PAH fraction as a whole is much more potent than the sum of the few PAH considered. Furthermore, humans are not exposed to individual PAH, but to PAH-containing mixtures. As a result, we have no knowledge of the human toxicity of individual PAH and the human data are available only for complex mixtures. We lack a *yardstick* by which the human toxicity of individual PAH could be estimated and thus the human risk must be projected indirectly from animal data.

An alternative approach is to estimate the potency of the PAH fraction as a whole. The second approach has been used by several risk assessment groups. It has an obvious merit of taking into account the *entire* PAH family of compounds. Furthermore, human health risk has been estimated for several PAH-rich mixtures and the results can be used as the basis for assessing the potency of other mixtures. Unfortunately, knowing the properties of a mixture obtained under certain conditions is no guarantee that a mixture obtained under another set of conditions will have similar properties.

PAH are emitted from a number of sources, which differ by factors such as the kind of fuel used, the amount of oxygen supply and the temperature of combustion. Furthermore, mixtures undergo photooxidation in the environment, and the PAH profile of the mixtures is altered in the process. PAH are usually particulate-bound in the environment. If different PAH are attached to different types of particles, their aerial transport may also be different. Therefore, the second approach which estimates the potency of the PAH fraction as a whole, though preferable, is dependent on whether one can predict the potency and composition of a mixture with reasonable accuracy. At the very least, one has to show that one can reasonably predict the potency or at least the composition of a mixture. We will examine this matter further when we discuss the *Whole Mixture Model* a little later.

Most existing risk assessment studies have used either one of the two approaches. The relative merits and the possible difference in the magnitude of the risk estimates of the two different approaches have not been critically reviewed. In addition, many of the assumptions behind the approaches have not been validated. Thus, even though a number of assessments are available, it is not clear how they compare and correlate with each other, and how reliable the assessments are.

Our goal, therefore, is not only to assess risk, but also to compare the two different approaches and to validate the assumptions on which the two models are based. We consider the validation and comparison of the two approaches a key element of our exercise. Our risk assessment is conducted using the two different models. This gives us the opportunity to juxtapose the results from the two risk assessments, compare and contrast them to the risk estimate determined from epidemiological studies. The following is a description of the two models we use.

- *Individual PAH model (IPM)*

  The IPM model estimates the risk of the PAH fraction of the mixture by summing the risks attributable to each compound present in the mixture. In order to use this model, environmental levels of individual PAH have to be determined. The risk attributable to each PAH can be determined as a product of its environmental level and its potency. Summing the risks attributable to individual PAH yields the risk of the PAH fraction.

- *Whole Mixture Model (WMM)*

  WMM is used to assess the risk of the PAH fraction of a mixture as a whole. The model assumes that the potency of the PAH fraction of any environmental mixture is proportional to the B[a]P content of the mixture. B[a]P thus serves as a surrogate for all other PAH and assumes the potency of the entire PAH fraction. In order to emphasize that we are not referring to just B[a]P but the whole PAH fraction, we define B[a]PS as a *B[a]P surrogate* that has the potency of the whole PAH fraction. The potency of the PAH fraction of a complex mixture is given by the product of the B[a]P content of the mixture and the typical potency of a PAH fraction of the mixture.

Sections 1.4.2 and 1.4.3 outline the conduct of the inhalation risk assessment using the two models as described. Section 1.4.4 describes how the risks due to oral and dermal exposure are derived from the risk associated with inhalation exposure.

1.4.2 Inhalation Potency of PAH Fractions: Estimates based on *Whole Mixture Model (WMM)*

Criteria for Use of Whole Mixture Model

In order to use the *Whole Mixture Model (WMM)*, we have to show that we can, with reasonable reliability, predict the levels of individual PAH in a complex mixture and the potency of the mixture based solely on its B[a]P content. In effect, we assume that the PAH profile[6] and the potency of the PAH fraction are reasonably similar from mixture to mixture.

In order to test our first assumption regarding the PAH profile of complex mixtures, we examined hundreds of mixtures, including both source mixtures (e.g coke oven emissions, coal tar, diesel emissions etc.), and environmental mixtures (air, soil and sediment in pristine, urban and heavily contaminated environment). We found that the differences in the PAH profiles of different mixtures are too small to have a significant effect on the potency of the mixtures.

Furthermore, we demonstrated that the complex mixtures rich in PAH (where PAH are the major contributors to the tumorigenic activity of the mixture) are roughly equipotent in tumorigenicity when the potencies are expressed in terms of their B[a]P levels. Although our conclusions are based primarily on rodent data, human epidemiological data are also consistent with the second assumption.

The verification that PAH-rich complex mixtures are approximately the same in both their PAH compositions and their potencies when expressed in terms of B[a]P levels, regardless of the source of the mixture, clears the way for the use of the *Whole Mixture Model (WMM)*.

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6 Levels of individual PAH relative to B[a]P level.

Assessment of Cancer Risk from Inhalation Exposure

On finding that the potencies of the PAH-rich complex mixtures are approximately the same when risk is expressed in terms of B[a]P content (based on epidemiological studies), we select the coke oven emissions study as the standard. The coke oven emissions study is chosen because it is based on relatively recent data of high quality. We then assume that the PAH fraction of the coke oven emissions is the principal contributor to the carcinogenicity of the emissions, and that the lung cancer risk of the PAH fraction is equal to the lung cancer risk of the coke oven emissions. The cancer risk from inhaling coke oven emissions in humans was estimated by USEPA in 1984 and was expressed as lifetime risk/(ng of B[a]P/m^3). Although we have selected this study as the standard for the potency of the PAH fraction, other risk assessment studies based on other data sets would have led to quantitatively similar results. We therefore define the *Mixture of Standard Potency (MSP)* as a mixture with cancer potency identical to that of the coke oven emissions estimated by USEPA (1984), or 2.3 x 10^{-5} ng B[a]PS/m^3.

In effect, we estimate that inhalation exposure to each additional ng of B[a]PS/m^3 of PAH fraction in air over a lifetime will increase the lifetime risk of lung cancer in humans by 2.3 cases per hundred thousand exposures.

Application

A risk assessor who wishes to apply the WMM model to estimate the lung cancer risk from exposure to the PAH fraction in air can multiply the average concentration of B[a]P in the air, expressed in ng B[a]PS/m^3, by 2.3 x 10^{-5}.

1.4.3 Inhalation Potency of PAH Fractions: Estimates based on *Individual PAH model (IPM)*

The *Individual PAH model (IPM)* estimates the risk of the PAH fraction of a mixture by summing the risks attributable to each individual PAH present.

Assessment of Lung Cancer Risk from Inhalation Exposure

The risk assessment of lung cancer risk from inhalation exposure to PAH in the environment consists of several distinct steps as outlined below.

- estimation of the inhalation potency of B[a]P
- estimation of the inhalation potency of other PAH relative to B[a]P
- assessment of the levels of individual PAH in the air
- assessment of the risk attributable to each PAH at environmental levels
- summation of the risks attributable to individual PAH

The first two steps are described in the present report. The subsequent three steps are in effect an application of the IPM to a particular environmental situation.

The first step in applying the IPM model is to estimate the human cancer risk from inhaling B[a]P. Most authors have conducted the estimation by extrapolating from rodent data using surface area and body weight extrapolation methods. These extrapolation procedures assume that the potency of PAH in different species can be predicted from the relative body weight or surface area (body mass to the power of k, where k is a constant between 2/3 and 1) of the animals. These methods have been validated by different authors for different compounds, but to our knowledge, not for PAH.

Our analysis indicates that surface area and body weight extrapolations of PAH potency from one rodent species do not appear to be predictive of PAH potency in another rodent species. If they are not good tools for interspecies extrapolation among rodents, they are less likely to be reliable for extrapolation from rodents to humans. We therefore consider the relative potency approach as our means of extrapolation.

In the present report, we provide evidence indicating that the relative potency of PAH or PAH-rich mixtures is independent of the animal species and the route of administration. The same assumption also implies that the relative potency of individual PAH and PAH-rich mixtures is the same in humans and experimental animal models (see equation 1.4.3.1).

$$C = \frac{human\,risk(PAH1)}{exper.potency(PAH1)} = \frac{human\,risk(PAH2)}{exper.potency(PAH2)} = \frac{human\,risk(mixt.1)}{exper.potency(mixt.1)} \qquad \text{Equation 1.4.3.1}$$

Based on the established inhalation potencies of coke oven emissions in both humans and in one rodent assay, and using equation 1.4.3.1, we calculated the human lung cancer risk due to B[a]P by inhalation from the potency of B[a]P in the same rodent assay. We then estimated the inhalation potency of individual PAH in humans by multiplying the B[a]P human inhalation potency by the relative potency of the PAH to B[a]P which has been established in the rodent assay.

In order to estimate the relative potency of PAH to B[a]P, we have taken the following steps.

- We estimated the potencies of PAH relative to B[a]P in four rodent assays.
- Having confirmed that the relative potency of PAH was similar in the four assays, we combined the four data sets.
- If the relative potency for a PAH was available from more than one assay, only one value was selected based on a pre-established priority for the four assays.

Using the method described, we have established the cancer potency from inhalation in humans for 209 PAH.

Since various authors have used different variations of the IPM model for the risk assessment of environmental mixtures, and since the IPM model is in accordance with existing MOEE regulatory practices, we evaluate the health risk due to PAH in environmental mixtures using the IPM model. We then compare the IPM risk estimate with the WMM estimate.

1.4.4 Potency of PAH Fractions by Oral or Dermal Routes: Estimates Based on IPM and WMM

Our discussion, so far, has focused on the inhalation of airborne PAH-containing particulates. A larger source of PAH for most Ontarians, however, is their diet. Some Ontarians may also be exposed to high levels of PAH by dermal contact. Dermal absorption could happen to individuals who frequently garden in contaminated soil or swim in heavily contaminated lakes and rivers. We therefore attempt to estimate the health risk due to ingestion and dermal absorption of PAH. Review of the current literature has not located any risk assessment on dermal absorption of PAH. The assessments of human health risk from oral exposure to PAH are usually conducted based on extrapolation directly from a rodent study. We estimate that the species extrapolation approach carries a considerable degree of uncertainty. Firstly, most assessors have relied on a single rodent study by Neal and Rigdon (1967). Secondly, the extrapolation itself is difficult and often uses extrapolation methods, such as body weight and surface area extrapolation, which we have judged to be inappropriate for PAH.

Our risk assessment from oral exposure to PAH is based on the assumption that the relative sensitivity of humans to PAH exposure via the oral and inhalation routes would be similar to that in rodents. The experimental data are generally consistent with our assumption. Furthermore, we have been able to use a number of rodent studies to calculate the relative potencies of PAH. Thus, even though further experiments are required to validate our estimates, we believe that our estimates for the potency of PAH fraction in humans due to exposure by the oral route are more reliable than those obtained by extrapolations based on the Neal and Rigdon study. We have used the same strategy to estimate the potency of PAH in humans from dermal exposure.

The potencies of B[a]P and the PAH fraction by the oral route were calculated by multiplying the lung cancer risk from inhalation exposure (derived using the IPM or WMM models in the case of mixtures) by the relative potencies of B[a]P or B[a]PS via the oral and inhalation route (derived from other species) as shown in equation 1.4.4.1 below. The potencies of PAH for the dermal route of exposure were derived by similar methods.

$$B[a]P_{humans,route} = B[a]P_{humans,inhalation} \times \text{Conversion Factor}_{route,species} \qquad \text{Equation 1.4.4.1}$$

The results are summarized in table 1.4.4.1 for B[a]P and in table 1.4.4.2 for B[a]PS. Note that the potencies in table 1.4.4.2 are higher for B[a]PS than the corresponding values in table 1.4.4.1 for B[a]P. This disparity is because B[a]PS represents the potency of the whole PAH fraction (see section 1.4.1) while the potency of B[a]P represents the potency attributable to B[a]P only.

Table 1.4.4.1 Estimates of human cancer risk associated with different organ sites and different routes of exposure, expressed in (risk/ng of B[a]P)/day. B[a]P in this context is free from particulates.

Route	Site of Tumor		
	Stomach	Lung	Skin
Topical			1.2 E-7
Respiratory	1.4 E-7 or 2.4 E-7 (/ng/m^3)	8.8 E-7 or 1.5 E-6 (/ng/m^3)	
Oral	2.6 E-9	2.1 E-10	

Table 1.4.4.2 Estimates of human cancer risk associated with different organ sites and different routes of exposure, expressed in risk/ng B[a]PS/day. B[a]PS in this context is the PAH fraction of the mixture free from particulates.

Route	Site of Tumor		
	Stomach	Lung	Skin
Topical			2.0 E-6
Respiratory	2.2 E-6 or 3.7 E-6 (/ng/m^3)	1.4 E-5 or 2.3 E-5 (/ng/m^3)	
Oral	4.2 E-8	3.4 E-9	

The cancer risk for any given individual PAH compound can be determined as the product of the compound's relative potency to B[a]P and the potency of B[a]P administered by the same route. The human cancer risk for the best known, among the 209, PAH are presented in table 1.4.4.3.

Table 1.4.4.3 Human cancer potency of some of the 209 PAH.

Compound	CAS #	Rel. Pot.	Inhalation				Ingestion		Dermal
			Lung $(ng/day)^{-1}$	Lung $(ng/m^3)^{-1}$	GI Tract $(ng/day)^{-1}$	GI Tract $(ng/m^3)^{-1}$	Lung $(ng/day)^{-1}$	GI Tract $(ng/day)^{-1}$	Skin $(ng/day)^{-1}$
acridine	000260-94-6	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
anthanthrene	000191-26-4	2.8E-01	2.5E-07	4.1E-07	3.9E-08	6.6E-08	5.9E-11	7.4E-10	3.4E-08
benz[a]anthracene	000056-55-3	1.4E-02	1.2E-08	2.1E-08	2.0E-09	3.3E-09	3.0E-12	3.7E-11	1.7E-09
benz[a]anthracene,7,12-dimethyl-	000057-97-6	1.9E+01	1.7E-05	2.8E-05	2.7E-06	4.5E-06	4.0E-09	5.0E-08	2.3E-06
benz[e]aceanthrylene	000199-54-2	1.2E-01	1.1E-07	1.8E-07	1.7E-08	2.8E-08	2.5E-11	3.2E-10	1.5E-08
benz[l]aceanthrylene	000211-91-6	7.7E-01	6.8E-07	1.1E-06	1.1E-07	1.8E-07	1.6E-10	2.0E-09	9.5E-08
benzo[a]acridine	000225-11-6	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene	000050-32-8	1.0E+00	8.8E-07	1.5E-06	1.4E-07	2.4E-07	2.1E-10	2.6E-09	1.2E-07
B[a]PS*	-	1.6E+01	1.4E-05	2.3E-05	2.2E-06	3.7E-06	3.4E-09	4.2E-08	2.0E-06
benzo[b]fluoranthene	000205-99-2	1.1E-01	9.7E-08	1.6E-07	1.5E-08	2.6E-08	2.3E-11	2.9E-10	1.4E-08
benzo[b]naphtho[2,1-d]-thiophene	000239-35-0	2.8E-02	2.5E-08	4.1E-08	3.9E-09	6.6E-09	5.9E-12	7.4E-11	3.4E-09
benzo[c]acridine	000225-51-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[c]phenanthrene	000195-19-7	2.3E-02	2.0E-08	3.4E-08	3.2E-09	5.4E-09	4.9E-12	6.1E-11	2.8E-09
benzo[e]pyrene	000192-97-2	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[ghi]perylene	000191-24-2	1.2E-02	1.1E-08	1.8E-08	1.7E-09	2.8E-09	2.5E-12	3.2E-11	1.5E-09
benzo[j]fluoranthene	000205-82-3	4.5E-02	4.0E-08	6.7E-08	6.3E-09	* 1.1E-08	9.5E-12	1.2E-10	5.5E-09
benzo[k]fluoranthene	000207-08-9	3.7E-02	3.3E-08	5.5E-08	5.2E-09	8.8E-09	7.8E-12	9.8E-11	4.6E-09
benzo[rst]pentaphene	000189-55-9	1.1E+00	9.7E-07	1.6E-06	1.5E-07	2.6E-07	2.3E-10	2.9E-09	1.4E-07
cholanthrene,3-methyl-	000056-49-5	2.6E+00	2.3E-06	3.8E-06	3.7E-07	6.2E-07	5.5E-10	6.9E-09	3.2E-07
chrysene	000218-01-9	2.6E-02	2.3E-08	3.8E-08	3.7E-09	6.2E-09	5.5E-12	6.9E-11	3.2E-09
cyclopenta[cd]pyrene	027208-37-3	1.2E-02	1.1E-08	1.8E-08	1.7E-09	2.8E-09	2.5E-12	3.2E-11	1.5E-09
dibenzo[a,h]acridine	000226-36-8	1.1E-01	9.7E-08	1.6E-07	1.5E-08	2.6E-08	2.3E-11	2.9E-10	1.4E-08
dibenzo[a,h]anthracene	000053-70-3	8.9E-01	7.8E-07	1.3E-06	1.3E-07	2.1E-07	1.9E-10	2.3E-09	1.1E-07
dibenzo[a,h]pyrene	000189-64-0	1.2E+00	1.1E-06	1.8E-06	1.7E-07	2.8E-07	2.5E-10	3.2E-09	1.5E-07
dibenzo[a,j]acridine	000224-42-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
indeno[1,2,3-cd]pyrene	000193-39-5	6.7E-02	5.9E-08	9.9E-08	9.4E-09	1.6E-08	1.4E-11	1.8E-10	8.2E-09
phenanthrene	000085-01-8	6.4E-04	5.6E-10	9.5E-10	9.0E-11	1.5E-10	1.3E-13	1.7E-12	7.9E-11
pyrene	000129-00-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00

* = Not a single PAH, but a surrogate for all PAH.

1.4.5 Findings and Conclusions

We have estimated the human cancer risk from unit exposure to about 209 PAH by the oral, respiratory, and dermal routes. The human cancer risk from unit exposure to the PAH component of complex mixtures by the same three routes have also been calculated. The results are summarized in tables 1.4.4.1 to 1.4.4.3.

Our assessment of risk is derived from human data on PAH-rich mixtures and correlates well with the potency of PAH-rich mixtures in humans. In contrast, most authors have conducted their assessments using rodent data. Their estimates correlate less well with the available human risk data and tend to underestimate the risk to humans from exposure to PAH. Our estimate of lung cancer risk from inhalation of B[a]P is one to two orders of magnitude higher than those reported by other authors.

Our estimates of risk from PAH by oral exposure is more than an order of magnitude lower than those obtained by other investigators. Our estimate is based on the assumption that the potency of PAH by inhalation relative to their oral potency is the same in rodents and humans. Data of higher quality are required to increase the reliability of the estimates for the oral potency of PAH.

We are unable to find any references to a dermal risk assessment on PAH other than our own. Therefore, no comparison can be made.

We validated all major assumptions that we have used in our assessment using all available data. We also examined some of the assumptions used by other investigators. The difference between our estimates and the estimates of other investigators is likely due to the difference in the extrapolation methods in predicting human potency from potency in rodents. We have presented evidence that the surface area and body weight extrapolations used by most other investigators is inappropriate for PAH and could lead to errors of several orders of magnitude.

We estimated that the risk attributable to PAH in the ambient air and indoor air is expected to be less than 2.4% of the total lung cancer risk that was reported and **not** attributable to smoking and radon gas in homes. This estimate is compatible with the current views on the contribution of ambient air to cancer risk.

The comparison of the two models we used for the dose-response assessment (*Individual PAH model (IPM)* and *Whole Mixture Model (WMM)*) suggests that the IPM model underestimates the risk calculation of the WMM model by almost two orders of magnitude. The result is consistent with our predictions, since the IPM model takes into account the risk attributable to only a handful of speciated PAH, while the WMM calculates the risk due to the PAH fraction as a whole. However, because of the assumptions on which the WMM is built, when the PAH profile of a mixture falls outside of the expected boundary, the IPM approach is preferable to the WMM.

We conclude that existing lung cancer risk assessments based on the summation of risks attributed to individual PAH underestimate the actual risk by two orders of magnitude and possibly more. At the same time, the risk from oral exposure to PAH may have been overestimated by an order of magnitude.

Human populations vary in their sensitivity to the carcinogenic effects of PAH, probably by more than an order of magnitude. In addition, short-term exposure (administered over one or two days) is about ten times more effective in inducing cancer than the same total exposure occurring over several weeks or a longer period of time. The age and sex of the exposed population are unlikely to have any effect on the risk from exposure to PAH mixtures.

1.4.6 Use of Most Likelihood Estimate (MLE) versus Upper Confidence Limit (UCL)

Throughout the report, we have attempted to provide our best estimate in the form of MLEs (most likelihood estimates). UCL (upper confidence limit), on the other hand, is the upper 95% confidence interval estimate on the initial slope of the dose-response curve. The variation of the slope stems strictly from the goodness of fit of a linearized multistage function to the data. The variation is often a relatively small source of uncertainty and the uncertainty associated with expert judgment often overshadows the uncertainty due to the variation in the data within a data set.

UCL slope values and unit risks can be readily derived from the MLEs by multiplying the MLE slope by 15.6. This conversion factor is the ratio between the UCL and the MLE values for the lung cancer risk for coke oven workers as determined by USEPA in 1984.

1.5 Uncertainty Assessment

1.5.1 Introduction

One way of looking at risk assessment is to consider it a process designed to reduce the uncertainty about the estimation of the potency of a toxicant to a tolerable level. At the beginning of a risk assessment, for example, experiments may have shown that a given compound may be toxic to some animals. At this early stage, there may be a great deal of uncertainty about the risk of the toxicant to humans. The risk assessment process reduces this uncertainty step by step, until at the end of the assessment, the uncertainty is considerably less than at the beginning of the process.

There are two different types of uncertainty. One is mathematical uncertainty which is associated with the variability of the data and the numerical processing of the data. This type of uncertainty has long been recognized and addressed in risk assessments, but is probably less important than the second type of uncertainty in the overall picture.

The second type of uncertainty arises in every risk assessment and is caused by the necessity of repairing gaps in the data using judgment, in combination with missing, incomplete or unreliable data. It is this type of uncertainty, the uncertainty associated with professional judgment, that should be given the greatest degree of attention, as it is usually the largest source of error.

In our opinion, the most important source of uncertainty is the use of unvalidated assumptions, judgments and generalizations to replace analysis and interpretation of available data. It is often argued that only data meeting certain quality criteria should be used in risk assessments, but following such criteria can restrict considerably the available database, forcing the investigator to rely on poorly substantiated assumptions in conducting the assessment. **We believe that, in general, careful evaluation of existing (and even imperfect) data permits risk assessment to have a lower overall uncertainty than the substitution of data analysis by unvalidated assumptions.** Our assessment, therefore, often depends on imperfect or incomplete data to validate our assumptions. Our approach is associated with an uncertainty of its own, which cannot be evaluated numerically in most instances.

We attempt to evaluate the magnitude of the uncertainty of each major step in our dose-response assessment (detailed in section 1.5.2). The uncertainty is expressed in terms of the range within which we expect the estimate to fall. The units of the range are orders of magnitude.

1.5.2 Summary of Assessment

Section 1.5.2 summarizes the estimation of the uncertainty associated with our risk assessment on PAH.

Table 1.5.2.1 presents the results of the uncertainty analysis for the dose-response assessment. The uncertainty is expressed in terms of the range within which the authors would expect to find the **actual** value. The range, expressed in orders of magnitude, is itself a judgment and is not a statistical expression. Our results clearly indicate that the *Whole Mixture Model (WMM)* is associated with a much lower uncertainty than the *Individual PAH Model (IPM)*.

Our estimates of uncertainty cannot be compared to the statistical uncertainty reported by a number of authors, because unlike other authors, who put their emphasis on numerical variation, such as the fit of a dose-response function to the data, our analysis emphasizes the uncertainty associated with the assumptions and judgments made. Under most circumstances, we expect our estimates of uncertainty to be higher than statistical uncertainty.

In our opinion, the main sources of uncertainty in a risk assessment originate in the dose-response assessment. The uncertainty from exposure assessment, for the most part, only slightly modifies the uncertainty from the dose response assessment. Hence, in order to reduce the uncertainty surrounding the PAH risk assessment, it would be more fruitful to reduce the uncertainty in the dose-response assessment.

Table 1.5.2.1 Uncertainty associated with estimates of cancer potency in humans (in orders of magnitude).

Extrapolation	Inhalation Primary	Oral Primary	Dermal	Inhalation Secondary	Oral Secondary
WMM model	<<1	1-2	1-2	1	2
IPM model	3-4	4	4	4-5	5

1.6 Recommendation

1.6.1 Comparison of WMM and IPM models

Either the WMM or the IPM model can be used to estimate the cancer risk due to complex PAH-containing mixtures. However, we strongly recommend that both models be used where appropriate, because each model is based on a different set of assumptions and each model estimates the risk of a different component of the mixture. This section provides the rationale for the use of each risk assessment model under different sets of conditions (see section 1.6.2 for recommendations).

The IPM model estimates the total risk of a small component of a mixture consisting of the few PAH for which human risk has been estimated. In general, the risk attributable to these PAH represents only a proportion of the potency of the PAH fraction of the mixture and underestimates the actual risk due to the PAH in the mixture. On the other hand, the risk estimate covers only the specified compounds and can be applied equally well to mixtures where the composition of PAH deviates significantly from the mixture of standard composition as described in section 3.7.1. This application is the main advantage of the IPM model as compared to the WMM.

The WMM model estimates the risk attributable to the **whole** PAH fraction of a mixture. During the development of the model, it was assumed that the lung carcinogenicity of coke oven emissions was due exclusively to the PAH in the mixture. Although coke oven emissions are rich in PAH, they may also contain other carcinogens. As a result, some overestimation of risk due to the PAH fraction is likely, but we do not expect the overestimation to be large. Nevertheless, it is necessary to consider the possibility that a mixture has a considerably different PAH profile than the mixture of standard composition as described and defined in section 3.7.2. Under such circumstances, the estimate of risk arising from the WMM model may not be acceptable.

In conclusion, the IPM model estimates the risk due to the identifiable PAH only and the remaining PAH are not considered. Therefore, the IPM model is expected to significantly underestimate the risk attributable to all PAH in the mixture. In contrast, the WMM model will probably somewhat overestimate the risk attributable to PAH in a mixture, and it may approximate or underestimate the potency of the whole mixture. The risk obtained using the WMM model is expected to be higher than the risk estimates derived by the IPM model. Therefore, the *true risk attributable to PAH* in a given mixture will lie somewhere between the values derived from WMM and IPM models, and is likely to be much closer to the estimate based on the WMM model.

1.6.2 When to use WMM and IPM

We recommend the use of the WMM as the primary model in most circumstances, while concurrently collecting data to permit the use of the IPM model. The IPM model would be used only when the PAH profile of the analyzed mixture deviates unacceptably from that of the mixture of standard composition (MSC). This theoretical "standard mixture" has a typical PAH profile, representative of many ambient and source mixtures that we have examined as part of the present assessment. The acceptability would be determined as follows.

- For both the MSC and the test mixture, determine the *total risk* as shown in the example below. Use only those PAH for which risk values are available and for which the relative contents are available for both the test mixture and the MSC.
- If the ratio of the *total risks* for the two mixtures is less than 0.1 or greater than 10, use the IPM model, or else use the WMM model.

As an example, we will take the data presented in table 1.6.2.1. The table provides an estimate of the potency of the MSC and a hypothetical test mixture. The test mixture has a considerably different composition of PAH than the MSC. The result indicates a difference in the potencies of the two mixtures. Since the ratio of the total risk of the MSC to the risk of the test mixture is less than 0.1 (actually 0.089), the IPM model would be used to estimate the potency of the test mixture.

1.6.3 PAH Monitoring Lists

Table 1.6.3.1 contains the human cancer potency of B[a]P and B[a]PS, and can be compared to table 1.6.3.2, which lists the human cancer potencies of a subset of PAH from the master list. The subset consists of the best known PAH, mostly parent compounds and heterocyclics. Many of the substituted PAH discussed in the report are probably not present in appreciable quantities in the environment, but a monitoring program designed to identify and quantify the PAH in the environmental mixtures is needed to verify this assumption.

Table 1.6.2.1 Cancer risk associated with MSC and a hypothetical "test mixture".

Compound	Risk/ng/m^3	MSC		Test Mixture	
		Relative Content	Risk/ng B[a]P/m^3	Relative Content	Risk$_{PAH}$/ng B[a]P/m^3
Benzo[a]pyrene	1.5 E-6	1	1.5 E-6	1	1.5 E-6
Anthanthrene	4.1 E-7	0.31	1.3 E-7	10	4.1 E-6
Anthracene	-	1.6	-	-	-
Benz[a]anthracene	2.1 E-8	1.2	2.5 E-8	1.2	2.5 E-8
Benzo[e]pyrene	0.0	1.1	0.0	0.0	0.0
Benzo[ghi]perylene	1.8 E-8	1.0	1.8 E-8	1.0	1.8 E-8
Benzofluoranthenes	9.4 E-8*	2.5	2.4 E-7	100	9.4 E-6
Chrysene/Triphenylene	3.8 E-8**	2.0	7.6 E-8	2.0	7.6 E-8
Coronene	-	0.34	-	-	-
Dibenz[a,h]anthracene	1.3 E-6	0.28	3.6 E-7	5.0	1.2 E-5
Fluoranthene	-	3.8	-	-	-
Indeno[1,2,3-cd]pyrene	9.9 E-8	0.86	8.5 E-8	0.86	8.5 E-8
Perylene	-	0.45	-	-	-
Phenanthrene	9.5 E-10	4.3	4.1 E-9	4.3	4.1 E-9
Pyrene	0.0	2.8	0.0	0.0	0.0
Acenapthylene	-	0.36	-	-	-
Acenapthene	-	0.71	-	-	-
Fluorene	-	1.6	-	-	-
Total			2.4 E-6		2.7 E-5

* Average of the b, j, and k isomers.

** Risk due to chrysene.

Table 1.6.3.1 Human cancer potencies for B[a]P and B[a]PS.

Name	CAS #	Rel. Pot.	Inhalation				Ingestion		Dermal Exp.
			Lung $(ng/day)^{-1}$	Lung $(ng/m3)^{-1}$	GI Tract $(ng/day)^{-1}$	GI Tract $(ng/m3)^{-1}$	Lung $(ng/day)^{-1}$	GI Tract $(ng/day)^{-1}$	Skin $(ng/day)^{-1}$
benzo[a]pyrene	000050-32-8	1.0E+00	8.8E-07	1.5E-06	1.4E-07	2.4E-07	2.1E-10	2.6E-09	1.2E-07
B[a]PS		1.6E+01	1.4E-05	2.3E-05	2.2E-06	3.7E-06	3.4E-09	4.2E-08	2.0E-06

In general, the PAH monitoring list should be designed according to the purpose of monitoring. Some of the options are discussed below.

- **Monitoring B[a]P alone**

  Monitoring B[a]P alone is sufficient to estimate the risk from exposure to the PAH fraction using the WMM. The use of the WMM implies, however, that the complex mixture being monitored has a PAH profile similar to that of the MSC. In order to verify that this condition holds, it is necessary to monitor also other PAH which constitute the MSC (see below).

  We recommend monitoring B[a]P alone under the following conditions.

  - once it has been shown that the PAH profile is sufficiently similar to MSC
  - as a pilot study to determine if there is a likely cause for health concern
  - in support of emergency actions

- **Monitoring carcinogens on the MSC list**

  The MSC list contains PAH with carcinogenic property and other PAH which do not appear to be carcinogenic. For the purpose of health assessment and in order to choose between the IPM and WMM, it is sufficient to measure the environmental levels of carcinogenic PAH. Carcinogenic PAH are defined, in the present report, as PAH compounds which have a relative potency (to B[a]P) higher than zero.

- **Monitoring all PAH on the MSC list**

  From human health perspective, it is not imperative that the remaining (noncarcinogenic) PAH on the MSC list be measured, since the levels of these compounds do not affect the cancer risk. This does not mean that the noncarcinogenic PAH should not be monitored for other reasons, and we do not discourage monitoring of these compounds.

Table 1.6.3.2 Relative potency and relative content of individual PAH (compared to B[a]P) in mixtures.

Compound	CAS #	Relative Content in MSC	Relative Potency
acenaphthylene	208-96-8	0.36	--
acridine	260-94-6	-	0.0E+00
anthanthrene	191-26-4	0.31	2.8E-01
anthracene	120-12-7	1.6	--
benz[a]anthracene	56-55-3	1.2	1.4E-02
benzo[a]acridine	225-11-6	-	0.0E+00
benzo[a]pyrene	50-32-8	1.0	1.0E+00
benzo[b]fluoranthene	205-99-2	2.5 - [b+j+k]*	1.1E-01
benzo[c]acridine	225-51-4	-	0.0E+00
benzo[c]phenanthrene	195-19-7	-	2.3E-02
benzo[e]pyrene	192-97-2	1.1	0.0E+00
benzo[ghi]perylene	191-24-2	-	1.2E-02
benzo[j]fluoranthene	205-82-3	2.5 - [b+j+k]*	4.5E-02
benzo[k]fluoranthene	207-08-9	2.5 - [b+j+k]*	3.7E-02
benzo[rst]pentaphene	189-55-9	-	1.1E+00
chrysene	218-01-9	2.0**	2.6E-02
coronene	190-07-1	0.34	--
cyclopenta[cd]pyrene	27208-37-3	-	1.2E-02
dibenzo[a,h]acridine	226-36-8	-	1.1E-01
dibenzo[a,h]anthracene	53-70-3	0.28	8.9E-01
dibenzo[a,h]pyrene	189-64-0	-	1.2E+00
dibenzo[a,j]acridine	224-42-0	-	0.0E+00
fluoranthene	206-44-0	3.8	--
fluorene	86-73-7	1.6	--
indeno[1,2,3-cd]pyrene	193-39-5	0.86	6.7E-02
perylene	198-55-0	0.45	--
phenanthrene	85-01-8	4.3	6.4E-04
pyrene	129-00-0	2.8	0.0E+00
pyrene,1-nitro-,	5522-43-0	-	0.0E+00

* sum of b, j, and k isomers.
** Chrysene/triphenylene

- not on the MSC list
-- potency data not available

- **Monitoring PAH to identify the source of emissions**

  Some PAH may be monitored so that the contribution of different types of emission sources to atmospheric PAH can be characterized. Coronene has been proposed, for example, as a marker for the mobile sources of PAH. Research in this area is desirable, but at present this approach is not ready for routine application.

- **Monitoring substituted PAH**

  Some evidence suggests that environmental mixtures contain a large number of substituted PAH and heterocyclics. Most of these compounds are not routinely monitored. Acquiring knowledge of the environmental levels for these compounds is desirable, if they were to be controlled in the future. However, the toxicity of many of these compounds is unknown and assessment of their human health implications may be difficult.

- **Monitoring PAH for which human toxicity has not been estimated**

  There may be compelling reasons to monitor PAH for which we have not estimated cancer potency. We do not discourage monitoring these compounds, but we suggest that a clear rationale for monitoring be established, since we will not be able to address questions regarding the human health effects of these compounds.

2 HAZARD IDENTIFICATION

2.1 Introduction

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental contaminants that are formed by the incomplete combustion of organic material, such as wood or fossil fuels. Studies with various mammalian species have shown that some PAH have a number of biologically toxic effects, including dermatological, ocular, immunological, reproductive, developmental, as well as carcinogenic effects. The carcinogenic effects of PAH have received the greatest scientific attention, as it is believed that they occur at doses lower than those required to produce the other toxic effects noted above. The main focus of this scientific criteria document will be on the carcinogenic effects of PAH. The purpose of the hazard identification section is to outline the physical and chemical properties of the PAH being studied and to provide a summary of the other biological effects attributed to PAH in the literature.

2.2 Physical and Chemical Properties of PAH

2.2.1 Basic Structure

Polycyclic aromatic hydrocarbons are made up of three or more fused benzene rings. Two basic types of PAH structures are possible: those where the carbon atoms at the fusion site (tertiary carbons) are centers for two linked rings (kata-annellated), and those where the tertiary carbons are the centers for three interlinked rings (peri-condensed) (Bjørseth and Becher, 1986; Zander, 1983). Kata-annellated PAH can be either straight chain compounds such as anthracene and pentacene, or angular compounds such as phenanthrene or picene (see figure 2.2.1.1). Some examples of peri-condensed PAH are shown in figure 2.2.1.2. Polycyclic aromatic hydrocarbons that contain five membered ring structures are also common (see figure 2.2.1.3). In addition to PAH composed only of carbon and hydrogen, some contain heteroatoms such as nitrogen (acridines) and sulphur (thiophenes).

The theoretical number of PAH is extremely large (Bjørseth and Becher, 1986; Zander, 1983). Twenty-two compounds can be generated, for example, by joining 5 benzene rings in various kata-annellated and peri-condensed configurations (Bjørseth and Becher, 1986). Eighty-two, 333 and 1448 compounds are possible using 6, 7 and eight rings respectively (Bjørseth and Becher, 1986; Zander, 1983). If 12 rings are used, then 683,101 theoretical compounds are possible (Bjørseth and Becher, 1986). It should be noted that these numbers do not include PAH with five membered ring structures or heterocyclic PAH. When these compounds are included, it is evident that the number of possible PAH becomes extremely large.

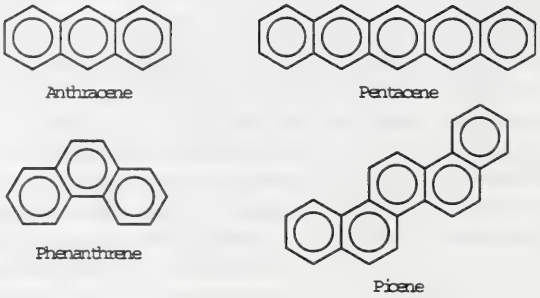


Figure 2.2.1.1 Straight chain and angular Kata-annellated PAH.

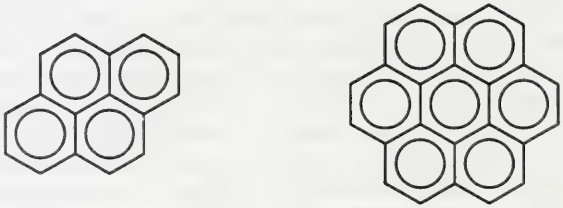
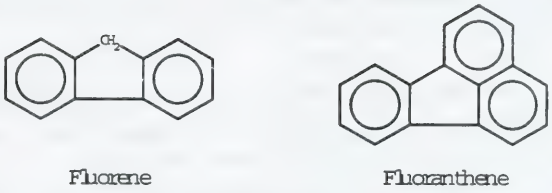


Figure 2.2.1.2 Peri-condensed PAH.



Fluorene Fluoranthene

Figure 2.2.1.3 PAH containing five-membered ring structures.

2.2.2 Physical and Chemical Properties

The physical and chemical properties of PAH are governed by their size (number of carbon atoms) and the shape (ring linkage pattern) of the π-electron system (Bjørseth and Becher, 1986; Zander, 1983). All completely unsaturated PAH are solid at room temperature and have relatively high melting and boiling points (Bjørseth and Becher, 1986; Zander, 1983). The physical and chemical properties of PAH have been extensively reviewed elsewhere (Bjørseth and Becher, 1986; Zander, 1983; USEPA, 1987; US Department of Health and Human Services (USDHHS), 1993; MacKay *et al.* 1992). This section is intended only as a summary of the information contained in these reviews. A list of structures and selected physical and chemical properties for a number of PAH are provided in table 2.2.2.1.

PAH are highly lipophilic and are essentially insoluble in aqueous systems (USDHHS, 1993). The high lipophilicity of PAH means that they tend to associate with particulate matter, humic matter or oily matter in aqueous systems. The aqueous solubility of PAH decreases with increasing molecular size. As a result, larger molecular weight PAH (≥4 rings) are almost exclusively bound to particulate matter, while lower molecular weight PAH (3 rings) can also be found in free water samples (USDHHS, 1993).

Vapor pressures for PAH are low and decrease with increasing molecular size; ranging between 1.6 E-2 Pa for phenanthrene and 2.0 E-10 Pa for coronene (Bjørseth and Becher, 1986). The low vapor pressure means that most PAH will be associated with particulate matter in the atmosphere (Bjørseth and Becher, 1986). Polycyclic aromatic hydrocarbons are thought to exist primarily in the vapor phase near the point of combustion (USEPA, 1987). As the gas stream is cooled, larger molecular weight PAH (≥ 4 rings) with lower vapor pressures condense onto particulate matter while the higher vapor pressures of the lower molecular weight PAH (3 rings) allow them to

remain partially in the vapor phase as well as to condense onto particulates (USEPA, 1987). As a result, in atmospheric samples, the larger molecular weight PAH are almost exclusively associated with particulate matter, while the lower molecular weight PAH can be found both free in the atmosphere and bound to particulates (USEPA, 1987).

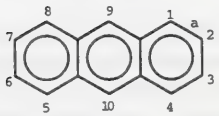
Table 2.2.2.1 Structures, physical and chemical properties of selected PAH.

For each PAH, a structure diagram is provided, indicating the correct orientation of the molecule according to IUPAC (see appendix E). Carbons 1 and 2 and face "a" have also been identified. Numbering for all compounds continues clockwise around the exterior of the molecules. Complete numbering has been provided for compounds where IUPAC numbering differs from the norm. Data on physical and chemical properties of PAH were from USDHHS (1993) and MacKay *et al.* (1992).

Anthanthrene

CAS #	191-26-4
Formula	$C_{22}H_{12}$
Molecular Weight	276.3 g/mol
Melting Point	264 °C
Boiling Point	-
Vapor Pressure	-
Density	-
Log Kow[7]	-
Solubility	-

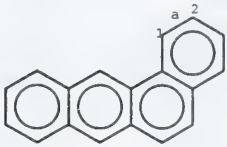
Anthracene



CAS #	120-12-7
Formula	$C_{14}H_{10}$
Molecular Weight	178.2 g/mol
Melting Point	218 °C
Boiling Point	342 °C
Vapor Pressure	0.001 Pa
Density	1.25 - 1.28 g/cm^3
Log Kow	4.4 - 4.5
Solubility	45 µg/L

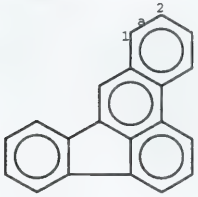
7 Log Kow = Log octanol/water partition coefficient

Benz[a]anthracene



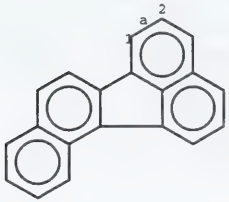
CAS #	56-55-3
Formula	$C_{18}H_{12}$
Molecular Weight	228.3 g/mol
Melting Point	167 °C
Boiling Point	435 °C
Vapor Pressure	2.8×10^{-5} Pa
Density	1.254 g/cm^3 @ 20 °C
Log Kow	5.6 - 5.9
Solubility	11 µg/L

Benzo[b]fluoranthene



CAS #	205-99-2
Formula	$C_{20}H_{12}$
Molecular Weight	252.3 g/mol
Melting Point	168.3 °C
Boiling Point	-
Vapor Pressure	-
Density	-
Log Kow	5.8 - 6.0
Solubility	1.5 µg/L

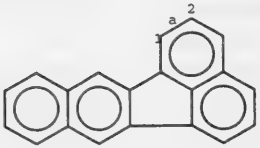
Benzo[j]fluoranthene



CAS #	205-82-3
Formula	$C_{20}H_{12}$
Molecular Weight	252.3 g/mol
Melting Point	165.4 °C
Boiling Point	-
Vapor Pressure	2.0×10^{-6} Pa
Density	-
Log Kow	6.76

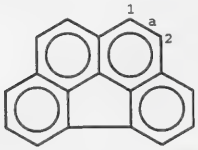
Solubility	2.5 µg/L

Benzo[k]fluoranthene



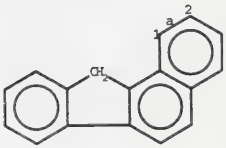
CAS #	207-08-9
Formula	$C_{20}H_{12}$
Molecular Weight	252.3 g/mol
Melting Point	215.7 °C
Boiling Point	480 °C
Vapor Pressure	5.8 x 10^{-8} Pa
Density	-
Log Kow	6.0
Solubility	0.8 µg/L

Benzo[ghi]fluoranthene



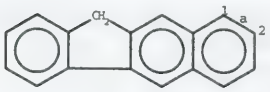
CAS #	203-12-3
Formula	$C_{18}H_{10}$
Molecular Weight	226.3 g/mol
Melting Point	149 °C
Boiling Point	-
Vapor Pressure	-
Density	-
Log Kow	-
Solubility	-

11H-Benzo[a]fluorene



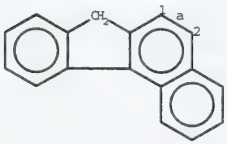
CAS #	238-84-6
Formula	$C_{17}H_{12}$
Molecular Weight	216.3 g/mol
Melting Point	189 - 190 °C
Boiling Point	398 - 400 °C
Vapor Pressure	-
Density	-
Log Kow	5.4
Solubility	45 µg/L

11H-Benzo[b]fluorene



CAS #	243-17-4
Formula	$C_{17}H_{12}$
Molecular Weight	216.3 g/mol
Melting Point	208 - 209 °C
Boiling Point	401 - 402 °C
Vapor Pressure	-
Density	-
Log Kow	5.75
Solubility	2.0 µg/L

7H-Benzo[c]fluorene

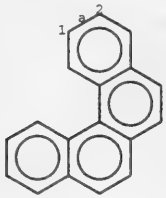


CAS #	205-12-9
Formula	$C_{17}H_{12}$
Molecular Weight	216.3 g/mol
Melting Point	124 - 125 °C
Boiling Point	-
Vapor Pressure	-
Density	-
Log Kow	-
Solubility	-

Benzo[ghi]perylene

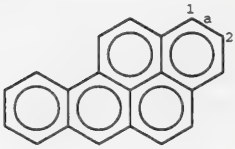
CAS #	191-24-2
Formula	$C_{20}H_{12}$
Molecular Weight	276.3 g/mol
Melting Point	278.3 °C
Boiling Point	-
Vapor Pressure	1.4×10^{-8} Pa
Density	-
Log Kow	6.5
Solubility	0.26 µg/L

Benzo[c]phenanthrene



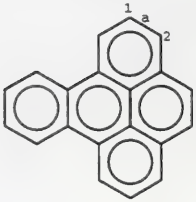
CAS #	195-19-7
Formula	$C_{18}H_{12}$
Molecular Weight	228.3 g/mol
Melting Point	66.1 °C
Boiling Point	-
Vapor Pressure	-
Density	-
Log Kow	-
Solubility	-

Benzo[a]pyrene



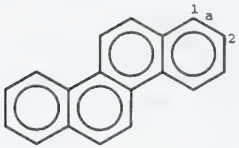
CAS #	50-32-8
Formula	$C_{20}H_{12}$
Molecular Weight	252.3 g/mol
Melting Point	179 °C
Boiling Point	310 - 313 °C
Vapor Pressure	7.0×10^{-7} Pa
Density	1.351 g/cm^3
Log Kow	6.1
Solubility	3.8 µg/L

Benzo[e]pyrene



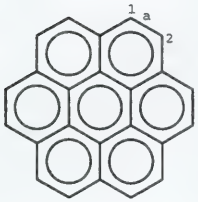
CAS #	192-97-2
Formula	$C_{20}H_{12}$
Molecular Weight	252.3 g/mol
Melting Point	178.7 °C
Boiling Point	310 - 312 °C
Vapor Pressure	7.4×10^{-7} Pa
Density	-
Log Kow	-
Solubility	4.0 µg/L

Chrysene



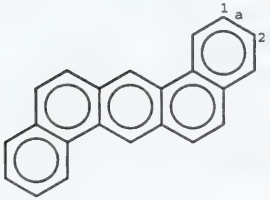
CAS #	218-01-9
Formula	$C_{18}H_{12}$
Molecular Weight	228.3 g/mol
Melting Point	255 - 256 °C
Boiling Point	448 °C
Vapor Pressure	5.7×10^{-7} Pa
Density	1.274 - 1.283 g/cm^3
Log Kow	5.61 - 5.86
Solubility	-

Coronene



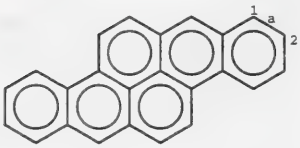
CAS #	191-07-1
Formula	$C_{24}H_{12}$
Molecular Weight	300.4 g/mol
Melting Point	438 - 440 °C
Boiling Point	525 °C
Vapor Pressure	2.0×10^{-10} Pa
Density	-
Log Kow	6.75
Solubility	0.14 µg/L

Dibenz[a,h]anthracene



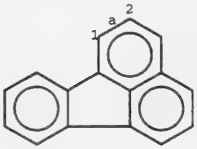
CAS #	53-70-3
Formula	$C_{22}H_{14}$
Molecular Weight	278.3 g/mol
Melting Point	262 °C
Boiling Point	269 - 270 °C
Vapor Pressure	3.7×10^{-10} Pa
Density	1.282 g/cm^3
Log Kow	6.75 - 6.84
Solubility	0.60 µg/L

Dibenzo[a,h]pyrene



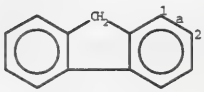
CAS #	189-64-0
Formula	$C_{24}H_{14}$
Molecular Weight	302.4 g/mol
Melting Point	317 °C
Boiling Point	-
Vapor Pressure	-
Density	-
Log Kow	-
Solubility	-

Fluoranthene



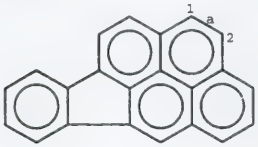
CAS #	206-44-0
Formula	$C_{16}H_{10}$
Molecular Weight	202.3 g/mol
Melting Point	111 °C
Boiling Point	375 °C
Vapor Pressure	0.00123 Pa
Density	1.252 g/cm^3
Log Kow	4.9 - 5.22
Solubility	260 µg/L

Fluorene



CAS #	86-73-3
Formula	$C_{13}H_{10}$
Molecular Weight	166.2 g/mol
Melting Point	116 - 117 °C
Boiling Point	295 °C
Vapor Pressure	0.090 Pa
Density	1.203 g/cm^3
Log Kow	4.18
Solubility	1900 µg/L

Indeno[1,2,3-cd]pyrene

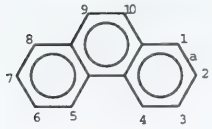


CAS #	193-39-5
Formula	$C_{22}H_{12}$
Molecular Weight	276.3 g/mol
Melting Point	163.3 °C
Boiling Point	524 - 534 °C
Vapor Pressure	1.3 x 10^{-8} Pa
Density	-
Log Kow	6.58
Solubility	62 µg/L

Perylene

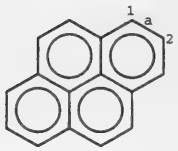
CAS #	198-55-0
Formula	$C_{20}H_{12}$
Molecular Weight	252.3 g/mol
Melting Point	273 - 274 °C
Boiling Point	-
Vapor Pressure	1.4 x 10^{-8} Pa
Density	-
Log Kow	6.25
Solubility	0.40 µg/L

Phenanthrene



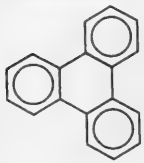
CAS #	85-01-8
Formula	$C_{14}H_{10}$
Molecular Weight	178.2 g/mol
Melting Point	100 °C
Boiling Point	340 °C
Vapor Pressure	0.020 Pa
Density	0.90 - 1.17 g/cm^3
Log Kow	4.45 - 4.57
Solubility	1100 µg/L

Pyrene



CAS #	129-00-0
Formula	$C_{16}H_{10}$
Molecular Weight	202.3 g/mol
Melting Point	149 - 156 °C
Boiling Point	385 - 404 °C
Vapor Pressure	6.0×10^{-4} Pa
Density	1.27 g/cm^3
Log Kow	4.88 - 5.18
Solubility	132 µg/L

Triphenylene



CAS #	217-59-4
Formula	$C_{18}H_{12}$
Molecular Weight	228.3 g/mol
Melting Point	197 - 199 °C
Boiling Point	425 - 436 °C
Vapor Pressure	2.3×10^{-6} Pa
Density	1.302 g/cm^3
Log Kow	5.49
Solubility	43 µg/L

2.2.3 Chemical Reactivity

Polycyclic aromatic hydrocarbons undergo substitution reactions with relative ease (USEPA, 1987). Oxidation and reduction reactions produce substituted PAH. The rates of electrophilic, nucleophilic, and free radical reactions tend to increase with increased conjugation (USEPA, 1987). Environmental factors such as temperature, light, oxygen levels, ozone levels, the presence of co-pollutants, and the particulate matter to which the PAH is adsorbed, all play a role in determining the chemical reactivity of PAH.

2.2.3.1 Photooxidation Reactions

Two types of oxidation reactions appear to be possible: photooxidations and chemical oxidations. The extended π-orbital system of PAH allows PAH to strongly absorb both visible and ultraviolet light (Bjørseth and Becher, 1986). As most PAH absorb light at the wavelengths found in sunlight (> 300 nm), it is likely that PAH are able to react through photo-induced chemical oxidations (Bjørseth and Becher, 1986). It has been suggested that photooxidation is probably one of the most important processes in the decomposition of PAH (Bjørseth and Becher, 1986; Zander, 1983; USEPA, 1987).

Most PAH are readily oxidized by air and sunlight (Bjørseth and Becher, 1986). The reactivity of pure PAH has been shown to be greater than that of PAH adsorbed to soot particles. This suggests that adsorption to particulate matter may provide some protection from photo-oxidation (Bjørseth and Becher, 1986). Specific rates for photooxidation have not been determined, but some studies suggest that the half-life for B[a]P in the presence of light and photochemical oxidants is in the order of hours to days (Bjørseth and Becher, 1986). Few studies have attempted to identify PAH photo-products in air, but those studies that have identified some products report the presence of endo-peroxides and quinone derivatives (Bjørseth and Becher, 1986).

2.2.3.2 Chemical Oxidations

Polycyclic aromatic hydrocarbons can undergo ozone and free radical oxidations (Bjørseth and Becher, 1986). The oxidation of B[a]P by ozone does not appear to be effected by irradiation (Bjørseth and Becher, 1986). Studies have shown that reactivity towards ozone depends upon the structure of the PAH (Bjørseth and Becher, 1986; Zander, 1983). Reactions with free radicals such as the hydroxyl radical have been studied in only a few cases. Studies have shown that certain PAH are rapidly oxidized in the dark when adsorbed to soot particles (Bjørseth and Becher, 1986). As soot particles have been shown to contain free radicals, it has been suggested that the "dark" oxidations may proceed through a free radical mechanism (Bjørseth and Becher, 1986; Zander, 1983).

2.2.3.3 Reactions with NO_x and SO_x

Studies have shown that PAH can react with NO_2, SO_2, and SO_3 when exposed to light and trace amounts of nitric and sulfuric acids (Bjørseth and Becher, 1986). The resultant products are nitro- and sulpho-substituted PAH derivatives (Bjørseth and Becher, 1986).

2.3 Absorption/Distribution/Excretion

The major routes of environmental exposure to PAH are through the lungs by the inhalation of aerosol or particulate-bound PAH, through the gut following the ingestion of contaminated water or food, and through direct dermal contact with PAH-contaminated dust, soil or sediments (IARC, 1983). Inhalation and oral uptake of PAH are the primary routes of exposure. Exposures through dermal contact are thought to represent only a minor exposure pathway for humans (USDHHS, 1993; IARC 1983). Absorption from all three routes has been extensively reviewed by both the International Agency for Research on Cancer (IARC, 1983) and the United States Department of Health and Human Services (USDHHS, 1993). The information contained here is a summary of the data provided in these documents.

The highly lipophilic nature of PAH means that they can easily cross the lipoprotein membranes of mammalian cells. Indeed, the fact that some PAH have been shown to produce toxic effects in organs removed from the PAH administration site confirms this (IARC, 1983; USDHHS, 1993). Once absorbed, PAH are rapidly distributed throughout the body (IARC, 1983). The lipophilic nature of PAH makes it difficult to eliminate them from the body without prior modification. The liver is the primary site of PAH metabolism, although a number of other tissues are also able to convert PAH to more water soluble products that can be removed from the body (see section 2.4). These PAH metabolites are removed from the body primarily in the bile and feces, and to a much lesser extent, the urine (IARC, 1983; USDHHS, 1993).

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

In the respiratory system, PAH are absorbed by transport across the mucous layer that lines the bronchi (USDHHS, 1993). The lipophilic nature of PAH allows them to cross the lung through passive diffusion and to partition into cellular lipids. Once absorbed, PAH are rapidly distributed throughout the body. The low PAH concentrations found in post-pulmonary blood indicates that diffusion across the lung wall is the rate-limiting step in the absorption process (USDHHS, 1993). Absorption rates vary between PAH and may be dependent upon their different solubilities in water (USDHHS, 1993).

In humans, the absorption of inhaled PAH has been inferred by the presence of various PAH metabolites in the urine of aluminum smelter workers exposed to PAH in the work place (USDHHS, 1993). In animals, studies of the absorption of inhaled PAH are limited to B[a]P. In rats, nose-only inhalation of radiolabelled B[a]P resulted in elevated tissue and excreta B[a]P levels within three hours of treatment. Intratracheally instilled radiolabelled B[a]P was found to be rapidly absorbed and distributed through the body, as indicated by the presence of the label in various tissues. Levels in the liver reached 21 percent of the administered dose within 10 minutes of treatment (USDHHS, 1993). Similar results were also reported for hamsters and guinea pigs (USDHHS, 1993).

Studies have indicated that the absorption of inhaled B[a]P can be affected by the size and nature of the particle to which B[a]P is bound (USDHHS, 1993; IARC, 1983). A study which monitored B[a]P clearance from the lung following intratracheal administration of either B[a]P crystals or B[a]P-coated carbon particles (0.5 μm - 1.0 μm and 15 μm - 30μm), found that 50 % of the pure B[a]P was cleared from the lung in 1.5 hours and that over 95% of the administered dose was cleared from the lungs within 24 hours. When coated onto carbon particles, B[a]P clearances were found to be much slower. For the smaller (0.5 -1.0 μm) particles, clearance of

50 percent of the administered dose required 36 hours, while for the larger (15-30 μm) particles, 50 percent clearance required 4 to 5 days (USDHHS, 1993). The results suggest that the bioavailability of B[a]P may be affected by particle size (USDHHS, 1993).

The available data indicate that inhaled PAH are absorbed across the wall of the respiratory tract, and that absorption may be affected by the water solubility of the individual PAH, as well as the size and nature of the particles to which they are bound (USDHHS, 1993; IARC, 1983).

2.3.1.2 Oral Exposure

In humans, evidence suggests that most of an ingested dose of PAH is absorbed across the gut wall (USDHHS, 1993). In animals, oral absorption has been shown to be incomplete and to be influenced by the presence of oils and fats in the gastrointestinal tract (USDHHS, 1993). In rats, for example, the presence of radiolabel in the liver, lung and kidney following an oral dose of[^{3}H]-B[a]P provides evidence that PAH are absorbed across the gut wall (USDHHS, 1993). In Sprague-Dawley rats infused duodenally with B[a]P for 90 minutes (total dose - 0.0005 mg/kg), absorption was estimated to be 40 percent. Oral absorption of PAH was measured in rats treated by gavage with high levels of B[a]P, anthracene or phenanthrene (dose not specified). Absorption of B[a]P was estimated to lie between 38 and 58 percent. Absorption of anthracene was found to be somewhat higher than that for B[a]P ranging from 53 to 74 percent, while phenanthrene was poorly absorbed (between 4 and 7 percent) (USDHHS, 1993).

Intestinal absorption of PAH has been found to be highly dependent upon the presence of bile. Bile aids in the solubilization of PAH, making them available for absorption. Studies have shown that the absorbed portion of a gastrointestinally instilled dose of B[a]P is absorbed during the uptake of fat soluble compounds (USDHHS, 1993).

2.3.1.3 Dermal Exposure

Evidence of the dermal absorption of PAH is available for humans. A study that applied crude coal-tar (2%) to human skin for 8 hours on two consecutive days reported that phenanthrene, anthracene, pyrene and fluoranthene were detected in the blood. Benzo[a]pyrene, also a constituent of coal-tar, was not detected (USDHHS, 1993). The failure to detect B[a]P was attributed to differences in absorption rates, tissue deposition and metabolism, conjugation and urinary excretion between B[a]P and the other PAH cited (USDHHS, 1993). *In vitro* studies with viable human skin suggest that substantial dermal metabolism and/or binding takes place with B[a]P and may limit the amount of B[a]P available to penetrate the skin (USDHHS, 1993).

Dermal absorption of B[a]P has been shown to be rapid in mice, rats, monkeys and guinea pigs (USDHHS, 1993; IARC 1983). Seven days after the application of a single dose of [^{14}C]-B[a]P (28 μg), approximately 73 percent of the administered dose was found to have been absorbed, the majority of which was absorbed within the first 3 days (USDHHS, 1993). Recovery of the radiolabel from feces, urine and tissues is an indication of dermal absorption (USDHHS, 1993). In rats, the percutaneous absorption of [^{14}C]-anthracene was estimated at 52.3 percent 6 days post-treatment. In guinea pigs, dermal absorption of [^{14}C]-phenanthrene and [^{14}C]-pyrene was found to be 80 and 90 percent respectively (USDHHS, 1993).

Data for both humans and animals show that PAH are absorbed through the skin. The mechanism of uptake is not known, but passive diffusion through the stratum corneum has been suggested as the most likely mechanism (USDHHS, 1993). The lipophilic nature of PAH would allow them to move easily into and through the outer layer of skin into the body (USDHHS, 1993; IARC 1983).

2.3.2 Distribution

Information regarding the distribution of PAH in humans following inhalation, oral or dermal exposure could not be located in the literature.

In animals, tissue distribution of B[a]P following inhalation exposure is qualitatively similar for different species (USDHHS, 1993). In rats, the lungs and liver were found to contain 2.7 percent and 4.6 percent of a recovered dose, six hours after intratracheal installation of 0.001 mg/kg [^{3}H]-B[a]P (USDHHS, 1993). Intratracheal installation of [^{3}H]-B[a]P in rats showed that the highest levels of radiolabel were found in the lungs, liver kidney, gastrointestinal tract and carcass (USDHHS, 1993). The concentration of B[a]P and metabolites in the small intestine increased with time, which suggests that biliary excretion and enterohepatic recirculation play an important role in the distribution of PAH and PAH metabolites (USDHHS, 1993). Mice that received intratracheal installations of B[a]P showed similar PAH distribution. The mice also experienced an increase in B[a]P levels in the pulmonary lymph nodes, however, as lung B[a]P levels decreased (USDHHS, 1993).

Orally absorbed benz[a]anthracene (B[a]A) and chrysene were rapidly and widely distributed in the rat (USDHHS, 1993). Maximum levels in perfused tissue such as the brain and liver were reached within 2 hours of treatment with high doses of B[a]A and chrysene (76 and 152 mg/kg respectively). Maximum levels in less perfused tissues such as adipose and mammary tissue were achieved within four hours of treatment (USDHHS, 1993). Fats from male Wistar rats treated with a single gavage dose of [^{14}C]-pyrene, were found to have the highest amount of label, followed by the kidney, liver and lungs (USDHHS, 1993). Dibenz[a,h]anthracene (DB[a,h]A) administered orally to female rats was found to be widely distributed throughout the body. However, maximal levels were not reached until ten hours post-treatment. The highest levels were found in the liver and kidney followed by the adrenal tissue, ovary, blood and fat. Elimination from the liver and kidneys was

found to be rapid and occurred 3 to 4 days post-treatment (USDHHS, 1993). DB[a,h]A was found only in the ovaries, fat and adrenal tissue (USDHHS, 1993). Pregnant rats were treated orally with 7,12-dimethylbenz[a]anthracene (DMBA), B[a]P or 3-methylcholanthrene (3-MC) (200 mg/kg) on day 21 of gestation. Within 30 minutes, DMBA could be detected in fetal tissue. Maximal fetal levels (1.5 -1.6 μg/g) were reached within three hours of treatment. Under the same conditions, maximal fetal B[a]P levels were found to be higher than DMBA levels (2.77 μg/g), while 3-MC was found in fetal tissue only in trace amounts (USDHHS, 1993).

Even though PAH are able to penetrate the skin, data on their distribution following dermal absorption is limited (USDHHS, 1993). A study that examined the distribution of [^{14}C]-anthracene following topical application to rats, found 1.3 percent of the applied dose in tissues (primarily liver and kidney) 6 days post-treatment (USDHHS, 1993).

Following absorption from any of the three routes considered, PAH are distributed throughput the body. There is insufficient information available to adequately characterize any differences which may exist in distribution between the three routes, but it would not be unreasonable to expect differences to exist. Orally absorbed PAH, for example, are liable to pass through the liver before being distributed throughout the rest of the body. Since the liver is the primary site of PAH metabolism (see section 2.4), it is likely that much of the absorbed parent PAH will be metabolized before being circulated or excreted. The levels of parent PAH leaving the liver could be expected to be significantly lower than the incoming levels, while the levels of metabolites leaving the liver could be expected to be higher. This effect is sometimes referred to as *first pass*. Unlike oral absorption, PAH entering the body via inhalation or dermal absorption do not undergo a *first pass* through the liver and are available for general circulation throughout the body. Therefore, while PAH are distributed through the body following absorption from all three routes, the pattern of compounds (parent and metabolite) and levels (amount available for general circulation compared to the amount of parent PAH absorbed) could be expected to differ between oral, respiratory and dermal absorption. Unfortunately, there is insufficient data available to establish the magnitude or significance of these differences.

2.3.3 Excretion

No quantitative data on the excretion of PAH following inhalation, oral or dermal exposure to PAH could be located for humans, but some qualitative data are available for human inhalation and oral exposure (USDHHS, 1993; IARC, 1983).

Studies have identified PAH and PAH metabolites in the urine of workers exposed to PAH in an aluminum plant, but a quantitative correlation between inhaled and excreted PAH could not be established from the data (USDHHS, 1993).

In rats, excretion of B[a]P following low level inhalation exposure has been reported to be both rapid and high (USDHHS, 1993). Fischer 344 rats were exposed to [^{14}C]-B[a]P (4.8 mg/m^3) through nose-only inhalation once a day over a period of four weeks. Excretion of radioactivity in the feces accounted for approximately 96 percent of the administered dose (USDHHS, 1993). Urinary excretion of radioactivity in rats following intratracheal installation of B[a]P accounted for 2.2 percent of the total dose 6 hours post-treatment (USDHHS, 1993). In dogs and monkeys, excretion of inhaled B[a]P was lower than that reported for rats. Less than ten percent of instilled radioactivity was excreted in the feces and urine of dogs and monkeys 48 hours after intratracheal installation with B[a]P (USDHHS, 1993).

Volunteers ingested diets high in PAH (0.007 - 0.02 mg/day) for two to three days. A 100 to 250 fold increase in B[a]P intake was found to correspond to an approximately ten fold increase in the elimination of 1-hydroxypyrene. Neither B[a]P nor its metabolites, however, could be measured in the excreta. The failure to detect B[a]P or its metabolites in the excreta makes it difficult to evaluate PAH excretion in humans following ingestion (USDHHS, 1993).

In male Wistar rats treated with a single dose of chrysene (0.22 mg/kg) by gavage, unmetabolized chrysene was found to represent 0.17 percent and 13.1 percent of the total dose in urine and feces 2 days post-treatment. Total dose recovery in excreta was 74 percent after four days. Metabolite levels in the feces were approximately 100 fold greater than those found in urine (USDHHS, 1993). A second study which treated male Wistar rats with single gavage doses of 2, 4, 6, 9 or 15 mg/kg of [^{14}C]-pyrene found that at the two lower doses, 82 percent of the administered dose was recovered in the excreta 2 days post-treatment. At the higher doses, total dose recovery in the excreta ranged between 50 and 63 percent (USDHHS, 1993).

Fecal elimination of B[a]P and B[a]P metabolites in Swiss Webster mice treated dermally with B[a]P (125 μg/cm^2) was found to account for 35 percent, 58 percent and 80 percent of the total recovered radioactivity 24 hours, 48 hours and 7 days post-treatment respectively. The amount of radioactivity found in the urine was approximately 10 percent of that found in the feces (USDHHS, 1993). Six days after the application of [^{14}C]-anthracene (9.3 μg/cm^2), the amount detected in the urine and feces was 29.1 and 21.9 percent, respectively (USDHHS, 1993).

The available data indicate that absorbed PAH are excreted both in the urine and feces. It also shows that hepatobiliary excretion and elimination through the feces are the major routes by which PAH are removed from the body, regardless of the route of PAH exposure (USDHHS, 1993; IARC, 1983).

2.4 Metabolism of PAH

Polycyclic aromatic hydrocarbons (PAH) are highly hydrophobic and essentially inert compounds that must be metabolically modified before they are sufficiently water soluble to be excreted (Gelboin, 1980). The process of transforming parent PAH into excretable products also generates reactive metabolites (Gelboin, 1980). These intermediates have the capacity to react with a variety of subcellular components such as protein and DNA (Estabrook *et al.* 1978). It is the binding of these intermediates to cellular macromolecules, particularly DNA, which is thought to be responsible for the biological activity of these compounds (Geacintov, 1988; Jankowiak *et al.* 1990). The metabolism of PAH can be broken down into two discrete and sequential stages identified as *Phase I* and *Phase II*. Both phases are controlled by a number of enzymes and both produce a multitude of products.

Phase I metabolic reactions increase the water solubility of a parent compound through an oxidation process that adds one or more hydroxyl groups to the parent core. These initial reactions are regulated by cytochrome P-450 (P-450) and epoxide hydrolase (EH) (Gelboin, 1980; Estabrook *et al.* 1978; Grover, 1986; Osborne and Crosby, 1987; DiPierre and Ernster, 1978; Cooper *et al.* 1983; Sims and Grover, 1981; Yuzuru *et al.* 1978; Guenthner and Oesch, 1981). The water solubility of these primary metabolites is greater than that of the parent compounds, but frequently is not great enough to ensure that it is removed from the system. As a result, these primary metabolites will either undergo further *Phase I* metabolic modification or be passed to *Phase II* for further processing.

Phase II metabolism increases the water solubility of the PAH metabolites by adding (conjugating) highly hydrophilic groups such as glutathione, or glucuronic or sulfuric acids (Cooper *et al.* 1983; Sims and Grover, 1981; Yuzuru *et al.* 1978). Most parent PAH are not sufficiently reactive to be suitable substrates for *Phase II* conjugating enzymes and must pass through *Phase I* metabolism before they can be processed by *Phase II*. However, some amino-, nitro- and hydroxy-substituted PAH are able to undergo *Phase II* conjugation without prior *Phase I* activation. While these latter compounds are able to sidestep *Phase I* metabolism, many still pass through Phase I reactions before being processed in *Phase II* metabolic modifications.

Although PAH vary greatly in size, ring configuration and substitution patterns, most undergo similar reactions in *Phase I* and *Phase II* metabolism and produce similar kinds of products. Differences arise in the location of the metabolic modifications and the reactivities of the intermediate metabolites formed. These differences play a major role in determining the biological activity of PAH. Most of the products of *Phase I* metabolism are readily conjugated by *Phase II* and excreted. A few PAH that possess a *Bay-Region* (see figure 2.4.1), can be converted to biologically reactive products if the initial oxidation reactions occur at or near the bay-region. The conversion of parent PAH to reactive metabolites and other products are discussed below.

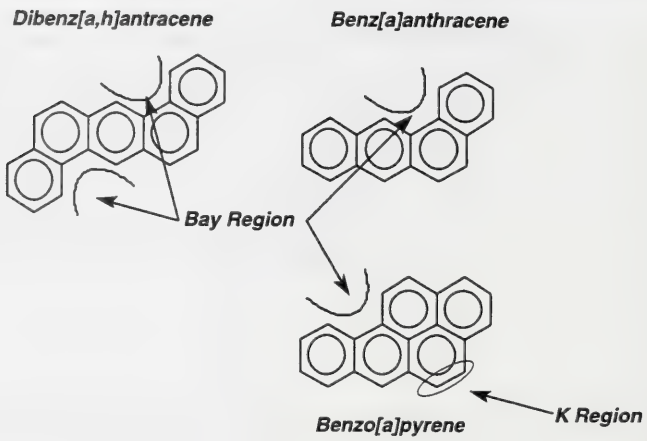


Figure 2.4.1 PAH Bay- and K- regions.

2.4.1 Phase I Metabolism

Phase I metabolism is governed by cytochrome P-450 and epoxide hydrolase. Together, these enzymes are responsible for the initial oxidations of PAH. Cytochrome P-450s are responsible for xenobiotic detoxification reactions in addition to the oxidation of numerous endogenous compounds including fatty acids, steroids, vitamins, and prostaglandins (Ryan and Levin, 1990). The identification of numerous cytochrome P-450 isozymes has helped to explain how this broad range of substrate specificity is achieved (Sims and Grover, 1981; Guengerich *et al.* 1982; Guengerich *et al.* 1981; Vlasuk *et al.* 1982; Wang *et al.* 1983; Imaoka, 1986; Imaoka *et al.* 1986; Halpert, 1988; Wilson *et al.* 1987; Parkinson *et al.* 1983; Ryan and Levin, 1990; Christou *et al.* 1987). Over twenty hepatic isozymes have been identified in the rat, but the total number of isozymes that exist in any given tissue remains unknown (Ryan and Levin, 1990). Cytochrome P-450s have been found in many tissues including the liver, lungs, kidney, testis, skin and gut (DePierre and Ernster, 1978; Cooper *et al.* 1983; Sabourin *et al.* 1988; Steinberg *et al.* 1987). In general, hepatic tissue has the highest

levels of cytochrome P-450, which is in accordance with the liver's function as the primary site of xenobiotic detoxification (Steinberg *et al.* 1987). Cytochrome P-450 levels in extrahepatic tissues are generally about ten percent of those found in hepatic tissue (Steinberg *et al.* 1987).

Although many cytochrome P-450 isozymes have been identified, only a small number (cytochrome P-450 a through e)[8] are responsible for the metabolism of most xenobiotics (Parkinson *et al.* 1983; Ryan *et al.* 1979; Chui and Yang, 1986). These isozymes are present in limited amounts in tissues from control animals but are inducible by some xenobiotic insults (Parkinson *et al.* 1983; Ryan *et al.* 1979; Chui and Yang, 1986). Cytochrome P-450c is the major isozyme responsible for the metabolism of PAH (Ryan *et al.* 1979; Chui and Yang, 1986). The levels of cytochrome P-450c can be induced in response to exposure to PAH (Parkinson *et al.* 1983; Ryan *et al.* 1979; Ryan and Levin, 1990). Exposure to PAH such as B[a]P or 3-methylcholanthrene (3-MC) can produce twenty to thirty-fold increases in the levels of cytochrome P-450c (Ryan *et al.* 1979; Chui and Yang, 1986). Therefore, exposure to PAH can result in an increase in the levels of the enzymes responsible for their removal (Ryan *et al.* 1979; Ryan and Levin, 1990; IARC, 1983). However, it is the metabolic processing of some PAH which results in their conversion to biologically active compounds. Thus, induction of cytochrome P-450 by PAH also results in an increased facility to generate reactive intermediates.

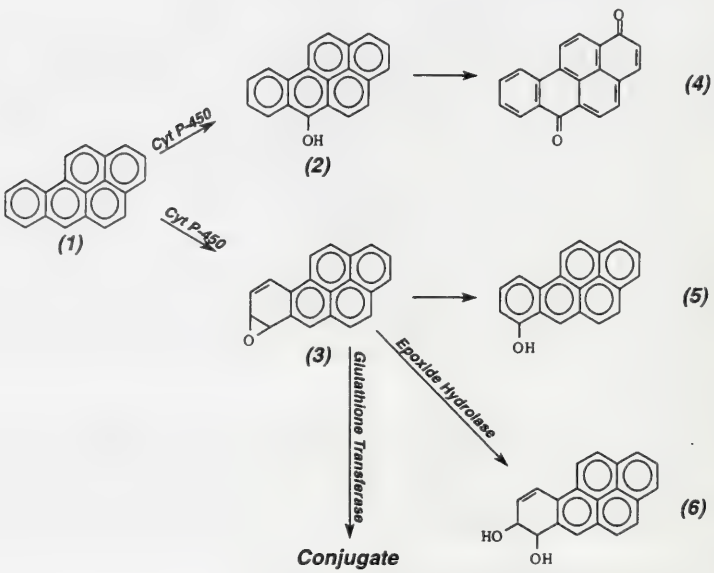
Many PAH undergo multiple *Phase I* processing reactions. It is possible to categorize these into primary and secondary reactions (Cooper *et al.* 1983). The initial oxidations which produce simple epoxides, phenols, quinones and dihydrodiols are considered primary reactions (Cooper *et al.* 1983), while subsequent *Phase I* reactions are considered to be secondary (Cooper *et al.* 1983). Both primary and secondary reactions and the products generated by each are considered below.

2.4.1.1 Primary Phase I Metabolism

Oxidation of the parent PAH molecule by cytochrome P-450 is the first step in the generation of most PAH metabolites. Cytochrome P-450-mediated reactions usually result in the addition of an oxygen atom across a double bond on the PAH to produce an arene oxide (primary epoxide, see figure 2.4.1.1.1) (Estabrook *et al.* 1978; Guenthner and Oesch, 1981; DePierre and Ernster, 1978) . At any given site, two epoxides can be formed which differ only in the stereochemical orientation of the epoxide to the PAH core (see figure 2.4.1.1.2). While

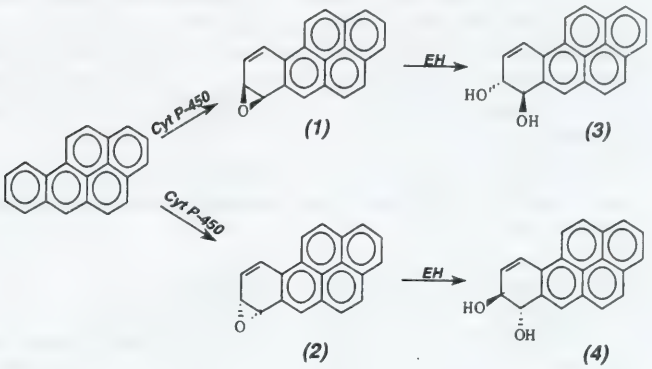
8 Labelling cytochrome P-450 isozymes has not yet been standardized. A multitude of nomenclature formats exist in the current literature. The present document will use the identification scheme employed by Ryan and co-workers because it is the simplest to follow (Ryan and Levin, 1990). While this system does not identify all of the cytochrome P-450 isozymes, it does identify twelve of the major cytochrome P-450 isozymes found in hepatic and extrahepatic tissues. These isozymes are distinguished by the use of lower case letters following the P-450: P-450a, P-450b, P-450c, P-450d, P-450e, P-450f, P-450g, P-450h, P-450i, P-450j, P-450k and P-450p. Although this system is not as descriptive as some of the systems currently in the literature, it is frequently used as a benchmark by other groups. As a result, this system is a useful means of comparing results between reports.

Figure 2.4.1.1.1 Primary ***Phase I*** metabolism of B[a]P (IARC, 1983).



	Compound	*CAS Number*	*Abbreviation*
1)	*Benzo[a]pyrene*	*50-32-8*	*B[a]P*
2)	*Benzo[a]pyrene, 6-hydroxy-,*	*33953-73-0*	*B[a]P-6-OH*
3)	*Benzo[a]pyrene, 7,8-dihydroxy-7,8-epoxy-, ±*	*64437-54-3*	*B[a]P-7,8-E (±)*
4)	*Benzo[a]pyrene, 1,6-quinone-,*	*3067-13-8*	
5)	*Benzo[a]pyrene, 7-hydroxy-,*	*37994-82-4*	*B[a]P-7-OH*
6)	*Benzo[a]pyrene, 7,8-dihydroxy-7,8-dihydro-, trans-, ±*	*61443-57-0*	*B[a]P-7,8-diol (±)*

Figure 2.4.1.1.2 Stereochemistry of epoxide and diol formation (IARC, 1983).



	Compound	CAS Number	Abbreviation
1)	*Benzo[a]pyrene, 7,8-dihydro-7β,8β-epoxy-, (+)*	*68906-82-1*	*B[a]P-7,8-E (+)*
2)	*Benzo[a]pyrene, 7,8-dihydro-7α,8α-epoxy-, (-)*	*68907-98-2*	*B[a]P-7,8-E (-)*
3)	*Benzo[a]pyrene, 7β,8α-dihydroxy-7,8-dihydro-, (-)*	*60864-95-1*	*B[a]P-7,8-diol (-)*
4)	*Benzo[a]pyrene, 7α,8β-dihyroxy-7,8-dihydro, (+)*	*62314-67-4*	*B[a]P-7,8-diol (+)*

studies have shown that cytochrome P-450 metabolism can occur at most, if not all, of the aromatic double bonds in a PAH molecule, reactions occur primarily at the periphery (Estabrook *et al.* 1978). At sites where there is sufficient electron density (C_6 of B[a]P, for example), cytochrome P-450 is also able to carry out oxidations at a single carbon to produce phenolic metabolites (Grover, 1986). These metabolites are, however, unstable and usually rearrange spontaneously to form quinones (see figure 2.4.1.1.1; Estabrook *et al.* 1978; Grover, 1986).

Once formed, primary epoxides are subject to a number of processes (see figure 2.4.1.1.1). They can undergo *Phase II* transformation and be are conjugated by glutathione transferase (GST) and excreted (Grover, 1986; Yuzuru *et al.* 1978). Spontaneous rearrangement of the primary epoxide yields phenolic metabolites which can be processed further in either *Phase I* or *Phase II* reactions (Cooper *et al.* 1983; Grover, 1986; Guenthner and Oesch, 1981). The primary epoxide can also be hydrated by EH to produce a dihydrodiol (diol) (see figure 2.4.1.1.1) (Grover, 1986). Because two stereochemically different epoxides can be produced at the same site on the PAH core, two diols are also produced, which differ only in the stereochemical arrangement of the hydroxyl groups (see figure 2.4.1.1.2) (Cooper *et al.* 1983). The significance of these stereochemical differences on the biological fate of the PAH metabolites is discussed in section 2.4.1.2.

Primary *Phase I* metabolism of classical PAH produces a mixture of dihydrodiols, phenols and quinones. For B[a]P, each of these compounds account for approximately 30 percent of the metabolites formed.

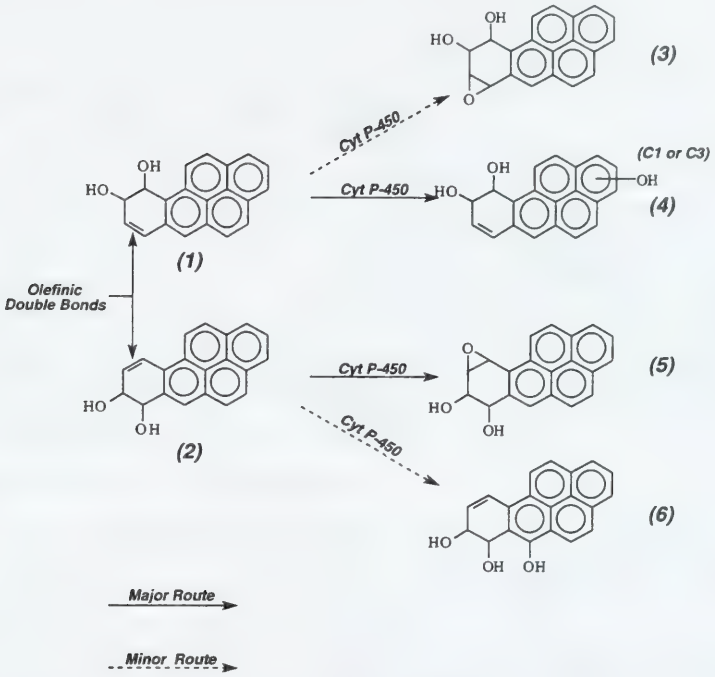
2.4.1.2 Secondary Phase I Metabolism

The products of secondary *Phase I* metabolism include diol-epoxides, triols, tetrols, and quinones (Cooper *et al.* 1983; Estabrook *et al.* 1978; Grover, 1986; Guenthner and Oesch, 1981; Yuzuru *et al.* 1978; Yang *et al.* 1978). The products formed by secondary *Phase I* metabolism are governed by the location where the substituents have been added to the parent compound by the primary reactions. The formation of each of the secondary products is discussed below.

2.4.1.2.1 Formation of Dihydrodiol-Epoxides

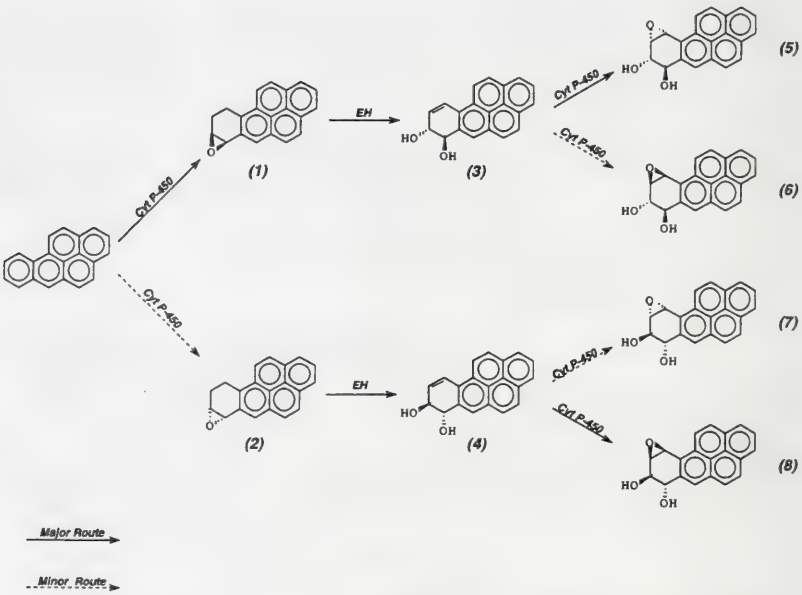
The formation of primary non-K-region diols is significant because these metabolites contain isolated olefinic double bonds in positions that allow the formation of dihydrodiol-epoxides (diol-epoxides) (see figure 2.4.1.2.1.1). Diol-epoxides are of concern because the epoxides of some PAH are readily converted into carbonium ions which can act as efficient alkylating agents (Jerina *et al.* 1977; Lehr *et al.* 1982). It is this alkylating potential which enables these compounds to bind to cellular macromolecules such as proteins or DNA to form adducts. It is the ability of these compounds to bind to DNA which is thought to be responsible for the biological activity of these compounds, resulting in genotoxic damage and allowing them to act as initiators of carcinogenesis (Geacintov, 1988; Jankowiak *et al.* 1990; USDHHS, 1993).

Figure 2.4.1.2.1.1 Differential processing of B[a]P diols (IARC, 1983).



	Compound	CAS #	Abbreviation
1)	*Benzo[a]pyrene, 9,10-dihydroxy-9,10-dihydro-, trans-, (±)*	*58030-91-4*	*B[a]P-9,10-diol (±)*
2)	*Benzo[a]pyrene, 7,8-dihydroxy-7,8-dihydro-, trans-, (±)*	*61443-57-0*	*B[a]P-7,8-diol (±)*
3)	*Benzo[a]pyrene, 9α,10β-dihydroxy-7β,8β-epoxy-7,8,9,10-tetrahydro-, (±)*	*58116-22-6*	
4)	*Benzo[a]pyrene, (1 or 3), 9,10-trihydroxy-*		
5)	*Benzo[a]pyrene, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro- (±)*	*55097-80-8*	*BPDE (±)*
6)	*Benzo[a]pyrene, 6,7,8-trihydroxy-,*	-	

Figure 2.4.1.2.1.2 Stereochemical metabolism of B[a]P-7,8-epoxide (IARC, 1983).



	Compound	CAS #	Abbreviation
1)	Benzo[a]pyrene, 7,8-dihydro-7β,8β-epoxy-, (+)	68906-82-1	B[a]P-7,8-E (+)
2)	Benzo[a]pyrene, 7,8-dihydro-7α,8α-epoxy-, (-)	68907-98-2	B[a]P-7,8-E (-)
3)	Benzo[a]pyrene, 7β,8α-dihydroxy-7,8-dihydro-, (-)	60864-95-1	B[a]P-7,8-diol (-)
4)	Benzo[a]pyrene, 7α,8β-dihyroxy-7,8-dihydro, (+)	62314-67-4	B[a]P-7,8-diol (+)
5)	Benzo[a]pyrene, 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+)	63323-31-9	(+)-BPDE-II
6)	Benzo[a]pyrene, 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro-, (-)	63357-09-5	(-)-BPDE-I
7)	Benzo[a]pyrene, 7α,8β-dihyroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+)	63323-29-5	(+)-BPDE-I
8)	Benzo[a]pyrene, 7α,8β-dihyroxy-9β,10β-epoxy-7,8,9,10-tetrahydro-, (-)	63323-30-8	(-)-BPDE-II

Diol location and conformation, and the selectivity of cytochrome P-450 appear to be the major determining factors in subsequent processing (Yang *et al.* 1978; Guenthner and Oesch, 1981). Secondary metabolism of B[a]P, 9,10-dihydroxy-9,10-dihydro- (B[a]P-9,10-diol), for example, is directed principally at C_1 or C_3 (see figure 2.4.1.2.1.1) with the formation of an epoxide group at C_7 and C_8 being only a minor route (IARC, 1983). The major metabolite of B[a]P, B[a]P-7,8-diol (±), is the corresponding B[a]P, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-, (B[a]P-7,8-diol-9,10-epoxide), with the formation of the B[a]P-6,7,8-triol being only a minor product (see figure 2.4.1.2.1.1 and IARC, 1983).

The conversion of B[a]P-7,8-E (±) to BPDE (±) yields four products which differ in the stereochemical arrangement of the hydroxyl and epoxide groups (see figure 2.4.1.2.1.2). Primary metabolism produces the (+) B[a]P-7,8-epoxide in a twenty-fold excess relative to the (-) B[a]P-7,8-epoxide (IARC, 1983). Both epoxides are converted to their corresponding diols (see figure 2.4.1.2.1.2). Further cytochrome P-450 activity produces the four diol-epoxides shown in figure 2.4.1.2.1.2. The predominant diol-epoxide formed is the (+) BPDE II (the full name for the compounds shown in figure 2.4.1.2.1.2 can be found in the footnote at the bottom of the figure). The significance of this to biological systems is that the predominant diol-epoxide has been shown to have the highest tumorigenic activity of the four diol-epoxides (IARC, 1983). It is also the predominant isomer found covalently bound to DNA in mammalian systems exposed to B[a]P (Jerina *et al.*, 1978).

2.4.1.2.2 Formation of Triols and Tetrols

Trihydroxy (triol) metabolites of PAH can be formed by two distinct mechanisms. The first involves the oxidation of phenolic metabolites, the second the hydrolysis of dihydrodiol-epoxides. The hydrolysis of dihydrodiol-epoxides to triol products is a non-enzymatic reduction that requires NADPH (Estabrook *et al.* 1978).

Tetrahydrotetrols are formed by the addition of H_2O across the epoxide of a dihydrodiol-epoxide compound (Estabrook *et al.* 1978). Because the addition can be either cis or trans, two distinct compounds can be produced from a single dihydrodiol-epoxide. A trans addition of H_2O across the 9,10-epoxide of BPDE-II will produce (7,10/8,9)-tetrahydrotetrahydroxyB[a]P, while the cis addition of H_2O yields (7/8,9,10)-tetrahydrotetrahydroxyB[a]P (Estabrook *et al.* 1978). This hydrolysis can be catalyzed by a number of acids including DNA (Estabrook *et al.* 1978).

2.4.1.2.3 Secondary Phase I Metabolism of Phenols

Cytochrome P-450 is capable of further oxidizing phenolic compounds to phenolic epoxides and quinones (Estabrook *et al.* 1978). Epoxides arising from this mechanism are

formed at sites removed from the original site of oxidation. Subsequent hydrolysis of the epoxide by EH yields a trihydroxy (triol) derivative (Estabrook *et al.* 1978). Both 3-hydroxy- and 9-hydroxy-B[a]P are metabolized, for example, to the corresponding B[a]P-4,5-epoxide. Hydrolysis of this epoxide produces 3,4,5-trihydroxy- and 4,5,9-trihydroxy-B[a]P respectively (Estabrook *et al.* 1978). Quinones are formed from phenolic metabolites if the initial hydroxyl group is in an appropriate location. For example, 3-hydroxyB[a]P can be converted to B[a]P-3,6-quinone (Estabrook *et al.* 1978).

2.4.2 Phase II Metabolism

There are a number of enzymes involved in the *Phase II* processing of PAH. Glutathione transferase (GST), Glucuronyl transferase (UDP-GT), and Sulphotransferase (ST) are the major enzymes that play a role in the conjugation of metabolites of classical, substituted and heterocyclic PAH (see table 2.4.2.1). The relative importance of any given enzyme pathway in the removal of PAH depends on the PAH being metabolized. For classical PAH, the myriads of metabolic products formed during *Phase I* mean that the *Phase II* enzymes will be responsible for the removal of a significant proportion of the metabolites formed. Substituted PAH often contain substituent groups which are suitable substrates for the various *Phase II* enzymes. These compounds can enter *Phase II* without prior activation by *Phase I*. The *Phase II* enzymes play a greater role in the removal of these compounds than classical PAH by removing parent compounds in addition to the metabolites formed by *Phase I* processes. A list of the enzymes, substrates and products of *Phase II* metabolism is provided in table 2.4.2.1.

2.4.2.1 Formation of Mercapturic Acids

Mercapturic acid derivatives of PAH are formed from GST-conjugated products. Glutathione-S transferase is responsible for transferring a glutathione moiety to an electrophilic receptor (Grover, 1986; Coles and Ketterer, 1990; Vos and Bladeren, 1990). The enzyme has a very wide substrate specificity (Grover, 1986; Coles and Ketterer, 1990). Primary epoxides and diol-epoxides are both able to serve as substrates for GST (Grover, 1986). Epoxides of varying complexity and molecular location differ in their ability to be conjugated by GST. Epoxides located in the K-region of a PAH are good substrates for GST, while non-K-region epoxides are poorer substrates (Grover, 1986; Nemoto, 1981). Phenols, diols and quinones are poor substrates because they do not possess the requisite electrophilic centers on the PAH molecules. The GST enzymes act directly upon the epoxide ring, opening it to produce a glutathione-PAH conjugate that is further processed by the removal of a glutamic acid and glycine residues from the glutathione, leaving a cysteine residue (Grover, 1986; Nemoto, 1981; Coles and Ketterer, 1990).

This cysteine is subsequently acylated to form the final mercapturic acid. Mercapturic acid products are more water soluble than the parent PAH and are excreted in the urine (Nemoto, 1981).

Table 2.4.2.1 Products of Phase II metabolism.

Substrate	Product	Enzyme	Reference
Primary epoxides	Mercapturic acids*	GST	1
Phenols	Sulphate esters	ST	2
Phenols	Glycosides**	UDP-GT	3
Quinones	Sulphate esters	ST	2
Quinones	Glycosides	UDP-GT	3
Dihydrodiols	Sulphate esters	ST	2
Dihydrodiols	Glycosides	UDP-GT	3
Triol-epoxides	Mercapturic acid	GST	1
Tetrahydrotetrols	Glycosides	UDP-GT	4
Amino substituted	Glycosides	UDP-GT	5
Amino substituted	Sulphate esters	ST	Nemoto, 1981
Nitro substituted	Glycosides	UDP-GT	5

* Glutathione transferases produce glutathione conjugates which are further processed to a final mercapturic acid.

** UDP-Glucuronyl transferases produce glucuronic acid conjugates which are further processed to yield glycoside products.

1 Grover, 1986; Yuzuru *et al.* 1978; Guenthner & Oesch, 1981

2 Grover, 1986; Yuzuru *et al.* 1978

3 Cooper *et al.* 1983; Yuzuru *et al.* 1978

4 Cooper *et al.* 1983; Osborne & Crosby 1987

5 Nemoto, 1981; Bock, 1991; Tephly *et al.* 1989; Burchell *et al.* 1991

2.4.2.2 Formation of Glycosides

Glycosides are formed from glucuronide conjugates of PAH metabolites. UDP-Glucuronyl transferase incorporates a glucuronic acid into a PAH metabolite producing a glucuronide, which is then processed to the final glycoside product (Nemoto, 1981). These glycoside products are excreted in both the bile and the urine (Grover, 1986). The UDP-GT enzymes are able to conjugate quinone, diol and phenolic metabolites of PAH (Nemoto, 1981). Examination

of UDP-GT activity towards metabolites of B[a]P has shown that phenols and quinones are preferentially removed, but UDP-GT activity towards B[a]P diols is lower (Cooper *et al.* 1983). The activity of UDP-GT towards diols was found to increase when the levels of UDP-GT were high (Cooper *et al.* 1983; Nemoto, 1981). Amino- and nitro- substituted PAH are sufficiently reactive so that UDP-GT is able to form conjugates with the substituent groups without prior *Phase I* activation (Nemoto, 1981, Bock, 1991, Tephly *et al.*, 1989, Burchell *et al.*, 1991). This capability, however, does not preclude these compounds from passing through *Phase I* transformation prior to conjugation (Nemoto, 1981). Substituted PAH that undergo *Phase I* metabolism are just as likely to be conjugated at the sites of *Phase I* activity than at the substituent sites (Nemoto, 1981).

2.4.2.3 Formation of Sulphate Esters

Sulphate ester derivatives of PAH are formed by ST which transfers a sulphate group to an appropriate nucleophilic center on the PAH molecule (Jakoby and Ziegler, 1990; Nemoto, 1981). Hydroxyl or amino groups are the most common recipients (Nemoto, 1981). Quinone and diol metabolites can also be conjugated by ST enzymes. Quinones must first be either partially reduced to semi-quinones or fully reduced to dihydroxy derivatives before they can be conjugated to sulphate esters by ST (Grover, 1986; Nemoto, 1981). Sulphate conjugates of diols appear as conjugates of catechols. This suggests that they arise through the dehydrogenation of a diol to a catechol (Grover, 1986; Nemoto, 1981). Therefore, ST activity towards diols is dependent on the ease with which the diol can be converted to the corresponding catechol (Grover, 1986; Nemoto, 1981). The rate of formation of sulpho-conjugates of B[a]P, 4,5-dihydroxy-4,5-dihydro (B[a]P-4,5-diol), for example, is three and eight times faster than that for B[a]P-9,10-diol and B[a]P-7,8-diol respectively (Nemoto, 1981). The water solubilities of the sulpho-conjugates are greater than those of the preconjugated metabolites, but not as high as those of the glucuronic acid conjugates (Nemoto, 1981).

2.4.3 Metabolism of Substituted PAH

Substituted PAH undergo many of the same reactions as classical PAH, but have the added potential of being metabolized at the substituent group (see table 2.4.3.1). Frequently, it is this side group metabolism which generates the final reactive product. Amino- and nitro- substituted PAH can be converted to aminoacyl intermediates that are capable of interacting with cellular macromolecules. The metabolism of each substituent group will be dealt with separately.

Table 2.4.3.1 Metabolites of substituted PAH.

Substrate	Product	Enzyme	Reference
Nitro-substituted PAH	Amino-substituted PAH	Nitro reductases	Rickert, 1987
Nitro-substituted PAH	Amino-substituted PAH	Non-enzymatic	Rickert, 1987
Nitro-substituted PAH	Ring oxidized PAH	Cytochrome P-450s	1
Amino-substituted PAH	N-Hydroxylated PAH	Cytochrome P-450s	2
Amino-substituted PAH	N-acetylated PAH	??	2
Amino-substituted PAH	Ring oxidized PAH	Cytochrome P-450s	3
Alkyl-substituted PAH	Hydroxyalkyl PAH	Cytochrome P-450s	4
Alkyl-substituted PAH	Ring oxidized PAH	Cytochrome P-450s	
Chloro-substituted PAH	Ring oxidized PAH	Cytochrome P-450s	
Hydroxy-substituted PAH	Ring oxidized PAH		

* Cytochrome P-450s indicates that more than one isozyme may be responsible for the formation of the product.

** S-Adenosylmethionine transferases

1 Rickert, 1987; Howard *et al.* 1988; Howard *et al.* 1985

2 Rosenkranz & Mermelstein, 1985

3 Rosenkranz & Mermelstein, 1985; Beland, 1989

4 Grover, 1986; Hecht *et al.* 1988; Christou *et al.* 1986; Bayless *et al.* 1986; Bao & Yang, 1986; Sullivan *et al.* 1982

2.4.3.1 Metabolism of Methyl-Substituted PAH

Methylated PAH are subject to metabolic attack both on the PAH core and on the methyl substituent. Cytochrome P-450 is able to carry out many of the common ring-oxidation reactions, producing epoxides which are converted to dihydrodiols, phenols and quinones (Grover, 1986; Hecht *et al.* 1988; Christou *et al.* 1986; Bayless *et al.* 1986; Bos *et al.* 1986; Sullivan *et al.* 1982). Dihydrodiols are further open to conversion to dihydrodiol-epoxides (Hecht *et al.* 1988; Christou *et al.* 1986; Bayless *et al.* 1986; Bos *et al.* 1986; Sullivan *et al.* 1982). Dihydrodiols, phenols and quinones are conjugated and excreted (Grover, 1986).

The location of the methyl substituent has a marked impact on the metabolism of PAH. The addition of a methyl group to C_5 of chrysene, for example, increases the tumorigenicity of the compound compared to the parent by facilitating the formation of a 1,2-dihydrodiol-3,4-epoxide (Hecht *et al.*, 1988). A methyl substitution at C_7 of B[a]P leads to the formation of 9,10-dihydrodiol-7-methyl-B[a]P, while a methyl substitution at C_{10} produces two epoxides: one at 4,5 and one at 7,8 (Sullivan *et al.* 1982).

Cytochrome P-450 is able to carry out a single electron oxidation of the methyl group to produce a hydroxymethyl group (Gelboin, 1980; Grover, 1986; Hecht *et al.* 1988; Bao and Yang, 1986). This has been proposed as the first step in an alternate activation pathway (Gelboin, 1980). Subsequent processing of the hydroxymethyl group by PAPS forms a methyl-sulphate ester. The sulphate moiety is a good leaving group and is easily removed to produce a benzylic carbonium ion (Gelboin, 1980; Grover, 1986; Christou *et al.* 1986; Bayless *et al.* 1986). It is this carbonium ion which is thought to be capable of binding to DNA and other cellular macromolecules (Gelboin, 1980; Grover, 1986; Christou *et al.* 1986). The importance of this reaction in the generation of reactive metabolites, however, is in question. Studies that have investigated the tumorigenicity of 5- and 6-hydroxymethylchrysenes have found that the tumorigenic potency of the 5-hydroxy compound was approximately equal to that of 5-methylchrysene, while the 6-hydroxy derivative showed little or no tumorigenic activity (Bao and Yang, 1986).

2.4.3.2 Metabolism of Amino-Substituted PAH

Amino-substituted PAH are subject to ring and N-oxidation reactions (Fu *et al.* 1982; Rosenkranz and Mermelstein, 1985; Beland, 1989). Ring-oxidation reactions generate similar metabolites to those produced from the parent PAH. Metabolic N-oxidation of amino-substituted PAH can follow either of two competing pathways: N-hydroxylation or N-acetylation (Fu *et al.* 1982; Rosenkranz and Mermelstein, 1985).

Ring-oxidation of amino-substituted PAH is influenced by the location of the amino group (Beland, 1989). The normal pattern of oxidation may be altered if the amino group is located at or near a site of normal ring epoxidation. Beland (1989) postulated that the increase in mutagenic activity observed in the Ames test for 4-aminobenzo[a]pyrene as compared to benzo[a]pyrene may be due to the presence of the amino group adjacent to a primary site of epoxidation. The blocking of this normal pathway could redirect metabolism to the benzyl ring region of the molecule, resulting in the increased formation of active 7,8-diol-9,10-epoxide B[a]P derivatives (Fu *et al.* 1982). Likewise, the observed reduction in Ames test mutagenicity of 6-, and 11-aminobenzo[a]pyrene as compared to B[a]P may be attributed to a reduction in the ring-oxidation on the benzylic ring of B[a]P as a result of the presence of an amino group adjacent to the site of epoxidation (Beland, 1989). Cytochrome P-450 oxidation can lead to the formation of ring hydroxylated products in which the hydroxyl group is located adjacent to the amino substitution. The presence of the amino group facilitates a positive charge delocalization over the ring and allows the enzymatic introduction of a hydroxyl group to take place either on the amino group or on a neighboring ring carbon (Fu *et al.* 1982).

N-hydroxylation of amino-substituted PAH mediated by cytochrome P-450, serves as an alternate activation pathway to ring-oxidation. N-hydroxylated products are proximate carcinogens. The final conversion to ultimate carcinogens requires a variety of post-oxidation enzymes including peroxidases and sulpho-, acyl-, aminoacyl-, and glucuronyl transferases (Fu *et al.* 1982). N-hydroxylated metabolites can also be converted to O-acetyl derivatives through acetyl-CoA dependent reactions. O-acetylated products can be converted into acetoxy esters which can bind to DNA (Rosenkranz and Mermelstein, 1985). The enzymes responsible for N-acetylation reactions are also able to carry out O-acetylation reactions (Rosenkranz and Mermelstein, 1985). The extent to which an aromatic amine is converted to a N-hydroxylated product depends upon the structure of the molecule and the cytochrome P-450 isozymes present (Fu *et al.* 1982; Rosenkranz and Mermelstein, 1985).

N-acetylation reactions can be considered true detoxification pathways. The formation of N-acetylated metabolites does not lead to the subsequent production of reactive metabolites. Further, their formation decreases the amount of aromatic amines available for N-hydroxylation (Rosenkranz and Mermelstein, 1985).

2.4.3.3 Metabolism of Nitro-Substituted PAH

The metabolism of nitro-substituted PAH has been extensively reviewed by Fu (1990). In mammalian systems, at least five pathways have been shown to lead to the production of potentially reactive metabolites (Fu *et al.* 1982; Howard *et al.* 1985, 1988; Rickert, 1987; Fu, 1990).

i) Nitro-reduction.
ii) Nitro-reduction followed by esterification.
iii) Ring-oxidation.
iv) Ring-oxidation followed by nitro-reduction.
v) Ring-oxidation followed by nitro-reduction and esterification.

Enzymatic reduction of the nitro group is thought to be an important activation pathway (Fu *et al.* 1982). The metabolic activation of nitro-substituted PAH has been proposed to involve ring-oxidation followed by nitro-reduction to produce ring oxidized N-hydroxylamino-PAH derivatives as penultimate products (Fu *et al.* 1982). In mammalian systems, these compounds have the potential to undergo further activation to acylated derivatives through reactions dependent on acetyl-CoA and mediated by O-acetyl-transferase (Fu *et al.* 1982).

Cytochrome P-450 is responsible for the ring-oxidation of nitro-substituted PAH (Fu *et al.* 1982; Howard *et al.* 1985, 1988; Rickert, 1987; Fu, 1990). Most mono-nitro-substituted PAH are detoxified by ring-oxidations to yield dihydrodiols, phenols, triols, and tetrols. These metabolites are further processed by *Phase II* enzymes before the compounds are finally excreted as glutathione, glucuronide or sulpho-conjugates (Howard *et al.* 1988). Nitro-substitution does

not appear to affect stereoselectivity, but does alter the regioselectivity of the oxidation reactions (Fu *et al.* 1982). The metabolism of 9-nitroanthracene to trans 3,4-dihydroxy-3,4-dihydro-9-nitroanthracene and of 7-nitrobenz[a]anthracene to its corresponding 3,4-dihydrodiol suggest that aromatic ring-oxidation is inhibited at positions located *peri* to the nitro group (Fu *et al.* 1982; Rickert, 1987). Formation of a dihydrodiol in positions *peri* to the nitro group, changes the conformation of the dihydrodiol from quasi-diequatorial to quasi-diaxial (Fu *et al.* 1982).

The nitro-reduction of nitro-substituted PAH is controlled by enzymes not normally associated with *Phase I* metabolism, including xanthine oxidase and alcohol dehydrogenase (Fu *et al.* 1982; Fu, 1990). Cytochrome P-450 has been shown to be capable of carrying out some nitro-reduction reactions in *in vitro* systems under anaerobic conditions (Rickert, 1987). Reduction of the nitro substituent is a three step process. Initially, the nitro group is reduced to a nitroso derivative. Subsequent processing further reduces nitroso intermediate through an N-hydroxylamino to a final amino-PAH product (Fu, 1990). Both the nitroso and N-hydroxylamino intermediates are potential electrophiles, but only the N-hydroxylamino appears to bind to DNA (Fu, 1990). While nitro-substituted PAH might be expected to undergo *Phase II* reactions without prior *Phase I* processing, this pathway does not seem to be the norm. Most nitro-substituted PAH pass through either ring-oxidation or nitro-reduction before they form conjugates in *Phase II*. However, some nitro-substituted PAH, (1-nitropyrene, 1-nitrobenzo[a]pyrene and 6-nitrochrysene) have been shown to significantly increase the levels of UDP-glucuronyl transferases and cytosolic glutathione transferases when administered intraperitoneally to rats (Fu, 1990). Sulphotransferases do not appear to be affected (Fu, 1990).

The reduction of nitro-substituted PAH can proceed by either enzymatic or non-enzymatic routes. Nitro-PAH can be reduced non-enzymatically by ascorbate, reduced pyridine and flavin nucleotides (Beland, 1989). Enzymatic reduction of nitro-substituted PAH produces nitrosoarenes, N-hydroxyarylamino-PAH and arylamines. Nitro and nitroxide radicals have also been detected (Fu *et al.* 1982). Nitrosoarenes and H-hydroxyarylamino-PAH can be further reduced enzymatically to arylamines by a number of enzyme systems throughout the cell (Fu, 1990). Arylamines produced in this manner may be passed on to the enzymes responsible for the metabolic removal of amino-substituted PAH.

2.4.3.4 Metabolism of Hydroxy-Substituted PAH

Hydroxy-substituted PAH are subject to the same reactions as classical PAH. The location of the hydroxy substituent can affect the metabolic profile, in that its location can dictate favourable and unfavourable steric processing. Because hydroxy-substituted PAH are normal products of classical PAH metabolism, these compounds will be processed in the same way as other phenolic and dihydrodiol PAH metabolites. They will be subject to further *Phase I* metabolism and to *Phase II* conjugation reactions.

2.4.3.5 Metabolism of Halo-Substituted PAH

The metabolism of halo-substituted PAH has not been studied to any great extent. The little information available seems to indicate that the halo substituent does not take an active part in the metabolic process. The location of the substituent can direct metabolic reactions either toward or away from reactive centers, depending upon their location on the core molecule. Metabolism of other halo-substituted organic compounds involves the halo group directly. These reactions are usually halo-substitution reactions in which the halo group is removed from the molecule and is replaced either by a hydrogen or hydroxyl group. These types of reactions do not seem to occur in PAH metabolism.

2.4.4 Metabolism of Heterocyclic PAH

The metabolism of heterocyclic PAH parallels that of classical PAH in many ways, as many of the same products are formed (see table 2.4.4.1). The presence of heterocyclic atoms, however, can have a marked effect on the observed metabolic profiles. Nitrogen heteroatoms are rarely sites of oxidation reaction, while sulphur heteroatoms are the predominant oxidation sites (Lehr *et al.* 1988; Jacob *et al.* 1988; Berthou and Vignier, 1986). Although nitrogen heteroatoms are not directly involved in oxidation reactions, their presence can direct oxidation reactions at other sites in a PAH molecule (Wood *et al.* 1986, 1989). The metabolism of PAH molecules containing nitrogen and sulphur is discussed below.

Table 2.4.4.1 Metabolic products of nitrogen- and sulphur-containing heterocyclic PAH.

Substrate	Product	Enzyme	Reference
Nitro heterocyclic PAH	Epoxides	Cytochrome P-450s	1
Nitro heterocyclic PAH	Dihydrodiols	Epoxide Hydrolase	1
Nitro heterocyclic PAH	Dihydrodiol-epoxides	Cytochrome P-450s	1
Nitro heterocyclic PAH	Phenols	Cytochrome P-450s	1
Nitro heterocyclic PAH	*bis*-Dihydrodiols	Cytochrome P-450s	Lehr *et al.* 1988
Sulpho heterocyclic PAH	Sulphoxide	Cytochrome P-450b	2
Sulpho heterocyclic PAH	Sulphone	Cytochrome P-450s	2
Sulpho heterocyclic PAH	Dihydrodiols	Cytochrome P-450s	Jacob *et al.* 1988
Sulpho heterocyclic PAH	Phenols	Cytochrome P-450s	Jacob *et al.* 1988

1 Lehr *et al.* 1988; Wood *et al.* 1986, 1989; Duke *et al.* 1988

2 Jacob *et al.* 1988; Berthou and Vignier, 1986

2.4.4.1 Metabolism of Nitrogen Heterocyclic PAH (Acridines)

Investigations of the metabolism of nitrogen-containing heterocyclic PAH have focused on acridines and their benzo- and dibenzo- derivatives (Lehr *et al.* 1988; Wood *et al.* 1986, 1989; Duke *et al.* 1988). The metabolic transformation of acridines appears to be similar to that of classical PAH. The major metabolites of unsubstituted acridines include epoxides, dihydrodiols, dihydrodiol-epoxides, phenols and *bis*-dihydrodiols (tetrols) (Lehr *et al.* 1988). The metabolism of methyl-substituted acridines such as 7-methylbenz[c]acridine also yield hydroxymethyl derivatives (Lehr *et al.* 1988). There is little evidence that the nitrogen heteroatom undergoes direct oxidation to any significant degree (Lehr *et al.* 1988). Furthermore, the formation of acridone (N=O) metabolites occurs only with acridines (Lehr *et al.* 1988).

Studies of the metabolism of benz- and dibenzacridines suggest that the formation of bay-region diol-epoxides are, at best, minor routes (Lehr *et al.* 1988). Rat liver microsomal metabolism of dibenz[a,j]acridine, however, produces extensive amounts of dibenz[a,j]acridine-3,4-dihydrodiol which has a bay region double bond and a K-region epoxide (dibenz[a,j]acridine-5,6-epoxide) (Duje *et al.* 1988). Respectively, these compounds account for 40% and 20% of the metabolites formed (Duke *et al.* 1988). The formation of dibenz[a,j]acridine-3,4-dihydrodiol is of concern because it is the precursor of the putative ultimate carcinogen dibenz[a,j]acridine 3,4-dihydrodiol-1,2-epoxide. The formation of a bay-region diol-epoxide from dibenz[a,j]acridine, however, is likely to be limited. Studies that have investigated the mutagenicity of dibenz[a,h]acridine and dibenz[c,h]acridine have found that the nitrogen heteroatom plays a role in the stabilization of the carbocation formed upon the opening of an epoxide ring (Wood *et al.* 1986, 1989). The formation of a carbocation is favoured when the epoxide ring opening takes place adjacent to the nitrogen heteroatom. Resonance delocalization of the positive charge is directed away from the nitrogen atom in these circumstances. When the epoxide ring is located on the opposite side of the ring from the nitrogen atom, delocalization of the carbocation charge results in an unfavorable charge on the nitrogen atom. The charge on the nitrogen atom may have a destabilizing effect that would prevent the formation of a bay-region diol-epoxide (Wood *et al.* 1986, 1989). This hypothesis is supported by the observed low activity of dibenz[c,h]acridine-3,4-dihydrodiol, a compound which has a bay region double bond open to diol-epoxide formation (Wood *et al.* 1986).

Phase II metabolism of nitrogen heterocyclic PAH can be expected to follow the same pathways as classical PAH, resulting in glutathione, glucuronide and sulpho-conjugate products.

2.4.4.2 Metabolism of Sulphur Heterocyclic PAH (Thiophenes)

The metabolism of sulphur-containing PAH (thiophenes) is dependent on the susceptibility of the sulphur atom to oxidation. Oxidation of the sulphur atom produces sulphoxides and

sulphones (Jacob *et al.* 1988; Berthou and Vignier, 1986). Ring-oxidation reactions produce epoxides, dihydrodiols, dihydrodiol-epoxides, phenols and quinones. Sulphone-phenolic products have also been identified, but it is unclear if the phenol formation precedes or follows the oxidation of the sulphur atom, or whether the order of oxidation is important at all (Jacob *et al.* 1988).

Investigations of thiophenes using rat liver microsomal preparations from control and treated animals have shown that the cytochrome P-450 system is able to produce many of the observed metabolites (Jacob *et al.* 1988; Berthou and Vignier, 1986). Microsomes from untreated rat liver can metabolize thiophene PAH analogues primarily through sulphur oxidation reactions to produce sulphoxides and sulphones (Jacob *et al.* 1988). Induction of cytochrome P-450b with compounds like DDT enhances sulphone formation. Induction of cytochrome P-450c by benzo[k]fluoranthene treatment increases ring-oxidation reactions leading to the formation of dihydrodiols (Jacob *et al.* 1988). Ring-oxidation reactions for thiophenes seem to be directed away from the K-region.

No information is available on the *Phase II* metabolic processing of thiophene metabolites. The sulphoxide and sulphone products may be sufficiently water soluble to be excreted without further processing. Given the requirement of a nucleophilic center for sulpho- and glucuronide transferases, it is unlikely that the sulpho-oxidized metabolites would serve as effective conjugate receptors. Ring oxidation products can be expected to be subject to *Phase II* processing in the same way as their classical PAH analogues.

2.5 Acute Toxicity

The effects of acute exposure to PAH in humans and animals has not been extensively investigated. A number of reports which provide some limited data are available. The most commonly reported data are LD_{50} (Lethal Dose: dose which kills half of a population in a given time). LD_{50} values are available for a number of PAH in multiple species and for various routes of administration (see table 2.5.1.1). Several other effects have also been reported. These effects include dermal irritation and photosensitization, ocular effects, changes in growth and DNA turnover, hepatic and renal effects, and are discussed below. Effects on the immune and reproductive systems following acute exposure to PAH have also been reported. As these systems appear to be important targets of PAH action, effects on these systems are considered separately (see sections 2.7 and 2.8). Much of the available acute toxicity data has been extensively reviewed by both the International Agency for Research of Cancer (IARC, 1983) and by the U.S. EPA (USEPA, 1991). The following is a summary of the data reviewed in these references.

2.5.1 LD_{50} Toxicity Data

IARC (1983), USEPA (1991) and USDHHS (1993) have all summarized the LD_{50} data currently available for PAH (see table 2.5.1.1). It is evident that LD_{50} values are available for only a limited number of PAH. In addition, route or species comparisons are difficult to make, since different studies cited had used different species, times and routes of administration. The only general statement that can be made is that the LD_{50} values for all the PAH shown in table 2.5.1.1 are high and all are in the g/kg range. There appears to be little species or route differences in LD_{50} values, but this finding cannot be adequately confirmed with the available data. No deaths have been reported in humans following inhalation, oral or dermal exposure (occupational or other) to PAH (USDHHS, 1993). Since environmental levels of PAH are much lower than those experienced in occupational settings, it is unlikely that short-term environmental exposures to PAH would cause death.

2.5.2 Respiratory Effects

Respiratory effects following acute exposure to PAH have been extensively reviewed by USDHHS (1993) and the Ontario Ministry of Labour (MOL, 1986). No studies have been located regarding respiratory effects in humans following inhalation exposure to individual PAH (USDHHS, 1993). Exposure to coal-tar pitch dust was noted to produce acute upper respiratory distress including nasal congestion, hoarseness, throat irritation and swelling (MOL, 1986). Workers exposed to asphalt that may have been adulterated with pitch reported symptoms including bronchitis, chronic cough, nose and throat inflammation and congestion (MOL, 1986).

2.5.3 Dermatological Effects

Dermatological effects resulting from skin contact with PAH have been reported in both experimental animals and humans. Skin contact with B[a]P, anthracene and PAH-containing complex mixtures such as coal-tar and coal-tar pitch have been reported to cause dermal irritation and photo-sensitization. The available data are discussed below.

Studies using animals have reported dermal irritation (Brune *et al.* 1978), photosensitization (Forbes *et al.* 1976) and alterations in the growth of epidermal tissue (Elgjo, 1968), following exposure to either B[a]P or anthracene. The application of 5 mg of B[a]P (0.5 ml of 1% B[a]P) to the intrascapular region of hairless mice produced a higher rate of epidermal growth than observed in non-treated animals (Elgjo, 1968). The results are difficult to interpret, however, as no solvent control was used. Brune *et al.* (1978) reported ID_{50} (dose which caused dermal irritation in 50 percent of the population) values of 0.015 mg/ear (56 nmoles/ear) and 0.12 mg/ear (660 nmoles/ear) for mice exposed to B[a]P and anthracene respectively. The effects reported in this study can be attributed to the test compounds, as appropriate controls appear to have been used.

Table 2.5.1.1 LD_{50} values for PAH.

Compound	LD_{50}*	Time	Species	Route	Reference
Acenaphthylene	3000		rat	oral	Knobloch, 1969
	2200		mouse	oral	Knobloch, 1969
Anthracene	> 430		mouse	ip	Salamone, 1981
Benzo[a]pyrene	≈ 250		mouse	ip	Salamone, 1981
Carbazole	> 500		rat	oral	Dieke *et al.* 1947
	> 5000	7 days	rat	oral	Eagle *et al.* 1950
Chrysene	> 320	24 hrs	mouse	ip	Simmon *et al.* 1979
Fluoranthene	> 500	7 days	mouse	ip	Gerarde, 1960
	≈ 2000	14 days	rat	oral	Smythe *et al.* 1962
	≈ 3200	24 hrs	rabbit	dermal	Smythe *et al.* 1962
Phenanthrene	700	24 hrs	mouse	ip	Simmon *et al.* 1979
Pyrene	510	7 days	mouse	ip	Salamone, 1981
	680	4 days	mouse	ip	Salamone, 1981

* - dose in mg/kg

Forbes *et al.* (1976) examined the photosensitizing effect of anthracene on the skin of hairless mice. Pretreatment of hairless mice with anthracene in a methanol carrier (4 μg anthracene/mouse, 0.16 mg/kg)[9] followed by exposure to ultraviolet (UV) light (λ > 290nm) produced a greater inflammatory response than that seen in animals treated with methanol alone, or animals that received anthracene but no UV exposure. The author suggested that the greater response in the animals which received both anthracene and UV exposure is evidence of the photosensitizing effect of anthracene. Anthracene has been shown to be photo-reactive and to undergo photo-induced oxidation reactions when exposed to sunlight (IARC, 1983; Paalme *et al.* 1983). Therefore it is possible that the pho-

9 Animal weights were not provided by the authors. However, if one assumes an average weight of 25 g/mouse, the dose of anthracene applied can be calculated as 0.16 mg/kg.

tooxidized products of anthracene, produced in the skin of the hairless mice upon exposure to UV irradiation, are responsible for the inflammatory response reported by Forbes *et al.*. Neither the mechanism by which the response is produced, however, nor the nature of the active compound(s) is known.

The effects of dermally applied B[a]P have been investigated in humans (Cottini and Mazzone, 1939). Cottini and Mazzone (1939) treated twenty-six patients with 120 daily applications of a solution of 1% B[a]P in benzene (treatment volumes were not provided). Evidence of elevated areas of hypertrophy of the papillae was observed in all patients. However, the total dose was not provided, nor was a benzene control used. Therefore it is not possible to determine if the observed effect was due to B[a]P, benzene or the action of both compounds.

The effects of exposure to coal-tar and coal-tar pitch in humans have been extensively reviewed in a report prepared for the Ontario Ministry of Labour (MOL, 1986). Crude coal-tar and coal-tar pitch were shown to be photosensitizing agents. Exposure to either mixture, followed by exposure to sunlight resulted in the immediate eruption of wheals and reddening, and subsequent epidermal injury (MOL, 1986). Photo-sensitization usually occurs within hours of exposure. Several such episodes may result in hyperpigmentation of the affected areas of the skin (MOL, 1986). Folliculitis, comedones and acneform lesions have also been reported following dermal exposure to either crude coal-tar or coal-tar pitch (MOL, 1986). However, these lesions usually occur only after prolonged exposure to high levels of the compounds (MOL, 1986).

2.5.4 Ocular Effects

Emmett *et al.* (1977) studied the effects of coal-tar pitch volatiles on rabbit eyes. Installation of 10 µl of coal-tar pitch volatiles distillate into the conjunctiva produced only minimal irritation, if the animals were not exposed to UV irradiation (Emmett *et al.* 1977). Exposure to longer wavelength UV radiation (λ = 330-380 nm) shortly after installation produced a marked photophobia and severe kerato-conjunctivity (Emmett *et al.* 1977).

In humans, acute exposure to coal-tar pitch dust or fumes produces a number of effects including the reddening of the eyelids and conjunctiva followed by distortion of vision and swelling and spasms of the eyelids (MOL 1986). Ocular effects usually begin two to four hours after the initial exposure (MOL 1986). In mild cases, the symptoms disappear within two to three days, but photophobia may persist for one to two months (MOL 1986).

2.5.5 Other Effects

Acute exposure to PAH has been reported to produce a number of other effects including alterations in growth rate, DNA turnover, as well as hepatic and renal function. Haddow *et al.* (1937) reported that a single ip injection of B[a]P (120 mg/kg) or DB[a,h]A (120 mg/kg) produced immediate

and persistent decreases in the growth of young rats compared to the control. Decreased growth rates in animals treated with DB[a,h]A persisted for at least fifteen weeks (Haddow *et al.*, 1937). Hellman *et al.* (1984) treated male C57BL mice with a single ip injection of B[a]P (28 mg/kg). A 40 percent reduction in DNA turnover occurred in the thymus, spleen, small intestine and testis 48 hours post-treatment. At the same time, DNA turnover rate increased in the liver (Hellman *et al.* 1984). The increase in DNA turnover was likely due to increased hepatic production of cytochrome P450c-associated enzyme activities (aryl hydrocarbon hydroxylase (AHH)). Benzo[a]pyrene has been shown to be an effective inducer of AHH (see appendix D). Studies have shown that induction of AHH activity by B[a]P and other PAH is achieved by *de novo* DNA, RNA and protein synthesis (see appendix D). This observation is consistent with the work of Asokan *et al.* (1986) who showed increased AHH levels in the hepatic and epidermal tissue of neonatal Sprague-Dawley rats twenty-four hours following a single topical application of fluoranthene (10 mg/kg). Yoshikawa *et al.* (1985) treated Sprague-Dawley rats with a single ip injection of phenanthrene (150 mg/kg). The liver and kidney appeared to be congested 24 and 72 hours post-treatment.

2.5.6 Summary

A number of effects resulting from acute exposure to PAH have been reported in the literature (see above). Of these reports, the determination of LD_{50} values and dermatological effects have received the greatest attention. Other effects, such as ocular, hepatic and renal effects, have received limited attention. The data available for any of these effects are very limited, so conclusions drawn must be viewed with caution. It does appear, however, that the doses required to produce many of these effects are very high (> 100 mg/kg), so it is unlikely that acute effects would be seen in humans at environmental exposure levels.

2.6 Chronic Toxicity

Information on the effects of chronic exposure to PAH in humans and experimental animals is limited. The available data have been extensively reviewed by IARC (1983), MOL (1986) and USEPA (1991). The reproductive and immune systems appear to be the primary targets of PAH action. Therefore, the effects of PAH on these systems are considered separately in sections 2.7 and 2.8. This section focuses on effects that do not involve the reproductive and immune systems.

Chronic occupational exposure to PAH fumes and PAH-carrying dusts has been reported to result in a number of respiratory effects including chronic bronchitis, pneumonitis and emphysema in humans (MOL, 1986).

The USEPA (1991) examined the effects of chronic oral exposure to a number of PAH on CD-1 mice. The mice were treated daily by gavage for 13 weeks with acenaphthylene (0, 100, 200 or 400 mg/kg/day), anthracene (0, 250, 500 or 1000 mg/kg/day), fluoranthene (0, 125, 250 or 500 mg/kg/day), fluorene (0, 125, 250 or 500 mg/kg/day), or pyrene (0, 75, 125 or 250 mg/kg/day). These studies were conducted over a number of years and not all effects were reported in all studies. Changes in red blood cell (RBC) count and liver weight were the only effects that were reported for all compounds. Other effects have been monitored for fewer PAH (see table 2.6.1).

Administration of acenaphthylene (0, 100, 200 or 400 mg/kg/day) produced a number of effects (see table 2.6.1). Decreased RBC count, increased liver weight and increased renal tubule regeneration compared to control were all reported at the lowest dose tested (100 mg/kg/day) and were found to be dose-dependent (USEPA, 1991). Increase in platelet count and decrease in hemoglobin content were reported but only at the 200 and 400 mg/kg/day dose levels. Increase in leucocyte count and hepatocellular hypertrophy were reported at the 400 mg/kg/day level only (USEPA, 1991). The lowest dose at which effects were reported was 100 mg/kg/day. Therefore the USEPA suggested that this value be considered the LOAEL (Lowest Observable Adverse Effect Level). Because the lowest dose tested produced effects, the USEPA was unable to establish a NOAEL (No Observable Adverse Effect Level) for acenaphthylene (USEPA, 1991).

No treatment-related effects were reported for mice treated with anthracene at any of the doses tested (USEPA, 1991) (see table 2.6.1).

Oral treatment of mice with fluoranthene at the 250 mg/kg/day dose level produced a decreased RBC count, mild nephropathy, a small increase in liver weight and an increase in SGPT (Serum Glutamic Pyruvic Transaminase) levels when compared to control (USEPA, 1991). Nephropathy was characterized by an increase in renal tubule regeneration. No other significant effects were reported at either the 250 or 500 mg/kg/day dose levels. Treatment at the lower dose (125 mg/kg/day) produced no observable effects. Based on these observations, the USEPA (1991) suggested that 250 mg/kg/day be considered to be the LOAEL and 125 mg/kg/day be considered to be the NOAEL.

Administration of fluorene (0, 125, 250 or 500 mg/kg/day) resulted in decreased RBC counts and increased liver weights, as compared to the control, at the 250 and 500 mg/kg/day dose levels (USEPA, 1991) (see table 2.6.1). Increases in kidney and spleen weights, relative to control, were reported at the 500 mg/kg/day level. Based on these data, the USEPA (1991) suggested a LOAEL of 250 mg/kg/day and NOAEL of 125 mg/kg/day for fluorene.

Table 2.6.1 Effects of chronic oral exposure to PAH in mice.

Effect	Acenaphthylene	Anthracene	Fluoranthene	Fluorene	Pyrene
RBC count	D[1] (100)[2]	N.C.[3]	D (250)	D (250)	D (250)
Hemoglobin	D (200)	N.C.			D (250)
Liver weight	I[1] (100)	N.C.	I (250)	I (250)	I (125)
Kidney weight		N.C.		I (500)	
Spleen weight		N.C.		I (500)	
Kidney path[4]	I (100)	N.C.	I (250)		I (75)
Liver path	I (100)	N.C.			
Platelet count	I (200)	N.C.			
Leucocyte count	I (400)	N.C.			
SGPT		N.C.	I (250)		
LOAEL[a]	**100 mg/kg/day**	**-**	**250 mg/kg/day**	**250 mg/kg/day**	**125 mg/kg/day**
NOAEL[a]	**-**	**-**	**125 mg/kg/day**	**125 mg/kg/day**	**75 mg/kg/day**

1 D = Decreased: I = Increased

2 Number in parentheses indicates the lowest dose at which effect is reported.

3 No change from control.

4 Increased renal tubule regeneration.

a LOAEL and NOAEL values are from USEPA (1991). Inclusion here should not be interpreted as either endorsement or acceptance of these values.

Oral treatment with pyrene produced a decreased RBC count and decreased hemoglobin levels when compared to control, at the 250 mg/kg/day dose level (USEPA, 1991). A statistically significant increase in liver weight, relative to control, was reported at the 125 mg/kg/day dose level. An increase in renal tubule regeneration was seen at the 75 mg/kg/day dose level. However, this increase was not considered to be significantly different from the control (USEPA, 1991). Therefore, USEPA (1991) suggested LOAEL and NOAEL values for pyrene of 125 mg/kg/day and 75 mg/kg/day, respectively.

The most common effects from chronic exposure to PAH appear to be decreases in RBC counts and increases in liver weights (see table 2.6.1). The mechanism by which the RBC count is reduced is unclear, and is not addressed by USEPA (1991). However, Robinson *et al.* (1975) showed that exposure to B[a]P resulted in a suppression of bone marrow activity and produced aplastic anemia and pancytopenia (see below). Therefore, it is possible that the decreased RBC counts result from the suppression of bone marrow activity due to PAH exposure. Increases in liver weight are likely due to increases in the levels of cytochrome P-450c in response to PAH exposure (see appendix D).

Robinson *et al.* (1975) examined the effect of B[a]P exposure on the lifespan of *responsive* C57BL/6 and C3H/HeN mice and *non-responsive* DBA/2 mice, which differ in their ability to metabolize PAH. Responsive mice have a greater capacity to metabolize PAH than non-responsive animals. A more detailed discussion of *responsive* and *non-responsive* animals, and the significance of this distinction can be found in section 2.7.1 and appendix D. The treated animals were fed chow which had been soaked in B[a]P-contaminated corn oil. The estimated daily dose was 120 mg/kg/day over a six month period. The survival of the non-responsive DBA/2 mice exposed to B[a]P containing chow was significantly lower than that seen in DBA/2 mice fed chow that contained just corn oil. At least half of the deaths in the DBA/2 treatment group occurred within the first fifteen days of the study. Death was attributed to the suppression of bone marrow activity, which resulted in aplastic anemia and pancytopenia (Robinson *et al.* 1975). Survival in the treatment groups of the two responsive strains of mice was not significantly different from their control groups (Robinson *et al.* 1975).

White and White (1939) fed pyrene (2000 mg/kg/day) to young rats for a period of 100 days. An inhibition of growth was observed and the livers were found to be enlarged and fatty in appearance. No other pathological conditions were reported.

Knobloch *et al.* (cited in USEPA, 1991) treated rats by gavage with acenaphthylene (600 mg/kg) in olive oil for ten days. This treatment was reported to produce "*significant*" body weight loss and changes in peripheral blood pattern and renal function (USEPA, 1991). Rotenberg and Mashbits (1965; cited in USEPA, 1991) exposed rats to acenaphthylene dust (0.5 and 1.25 mg/m^3) four hours per day for four months. After three weeks of treatment, a delay in weight gain and a tendency towards lower blood pressure was reported in the treated animals when compared with control (Rotenberg and Mashbits, 1965; cited in USEPA, 1991).

A study which administered anthracene to BDIII rats in the diet (15 mg/rat/day to a total dose of 4.5 g: approximately 550 days) showed no treatment-related adverse effects (Schmähl, 1955; cited in USEPA, 1991).

Hoch-Ligeti (1941) treated albino mice with weekly subcutaneous (sc) injections of dibenz[a,h]anthracene (DB[a,h]A, 0.25 mg/treatment; 10 mg/kg if a weight of 25 g/mouse is assumed) for 40 weeks. Between 20 and 30 weeks, increases in stem cells, accumulated iron and reduction in lymphoid cells were reported in the treated animals (Hoch-Ligeti, 1941). Spleen weights in the treated group were lower than those in the control group. In addition, liver and kidneys in the treated mice

showed signs of degeneration that were not reported in the control group (Hoch-Ligeti, 1941). Similar treatment with benz[a]anthracene showed no treatment-related effects in the liver, kidney or lymphoid tissue (Hoch-Ligeti, 1941).

Lasnitzki and Woodhouse (1944) treated male rats with 20 sc injections of DB[a,h]A (0.278 mg/treatment; 1.1 mg/kg if a weight of 250 g/rat is assumed) for a total dose of approximately 5.6 mg DB[a,h]A (22.4 mg/kg if a weight of 250 g/rat is assumed). In the treated group, lymph nodes underwent haemolymphocyte changes which included the presence of RBC in the extravascular spaces of the lymph nodes (Lasnitzki and Woodhouse, 1944).

The limited data available shows that chronic exposure to PAH may produce a number of effects. The effects most commonly reported are decreases in RBC count, weight gain and increases in liver weight. In general, these effects seem to occur only after exposure to reasonably high levels of PAH. The lowest dose at which effects were reported was 5.6 mg DB[a,h]A (22.4 mg/kg if a weight of 250 g/rat is assumed) (Lasnitzki and Woodhouse, 1944). The other doses reported ranged between 15 mg/rat/day (Schmähl; cited in USEPA, 1991) to 2000 mg/kg/day (White and White, 1939). USEPA (1991) recommended NOAEL values that range from 75 mg/kg/day (pyrene) to 125 mg/kg/day (fluoranthene and fluorene). It should be noted, however, that these values were derived for PAH that have relatively low carcinogenic potencies. The work of Robinson *et al.* (1975) on B[a]P indicates that chronic effects could be seen in responsive mice only at doses comparable to those reported above. This implication would suggest that PAH with moderate or high carcinogenic potencies are likely to produce chronic effects at doses similar to those reported here. However, the available data are insufficient to adequately characterize the effects due to chronic exposure to PAH.

2.7 Reproductive/Developmental Toxicity

The reproductive and developmental toxicity of PAH following inhalation or dermal exposure in humans and experimental animals have not been reported in the literature. A number of authors, however, have reported effects of PAH administered either orally or by injection. The majority of the information focuses on B[a]P, while a smaller number of reports provide data for other PAH and PAH-containing mixtures.

Reproductive and developmental effects that have been reported for PAH include fetal malformations, mortality, tumorigenicity, immunosuppression and clastogenicity, and effects on the adult male and female reproductive systems. The data relating to each of these effects are discussed below.

2.7.1 Fetal Mortality

Fetal mortality is the most common reproductive effect reported for PAH exposure resulting from both oral and injection routes of administration. The available data are discussed below.

Rigdon and Rennels (1963) maintained female rats (strain not specified) on a diet containing 1 mg of B[a]P per gram of food. In the control group, fifty percent of the females became pregnant (three of six) and all three delivered normal litters. Five of the eight animals in the test group became pregnant, but only one delivered a litter. Only half of the pups in this litter survived. The remaining animals were malformed and stillborn (Rigdon are Rennels, 1963). The data suggest that exposure to B[a]P in the diet affects breeding success by increasing fetal mortality. Since the final dosages could not be determined and the number of animals used was small, the significance of these findings is difficult to evaluate. MacKenzie and Angevine (1981) treated CD-1 mice daily with B[a]P by gavage on days 7 through 16 of gestation. Four test groups consisting of 30 to 60 animals were treated daily with 0, 10, 40 or 160 mg/kg body weight. No maternal toxicity was observed at any of the doses tested. The number of pregnant females and the number of viable litters produced was significantly lower in the 160 mg/kg dose group than in the other groups. In addition, the mean litter weights were lower for all test groups compared to control (0 mg/kg). These data suggest that maternal dietary exposure to B[a]P can affect breeding success by reducing the number of viable litters produced and by lowering litter weights.

Khan *et al.* (1987) monitored the effect of a PAH-containing mixture (Prudhoe Bay Crude Oil (PBCO)) on fetal mortality in Sprague-Dawley (SD) rats. Pregnant SD rats were treated with PBCO by gavage (1.0 ml or 2.0 ml) on days 6 through 17 of gestation. Animals treated at both doses showed significantly greater levels of fetal resorption than the controls, 4.5 and 11 fold respectively. Histological examination showed that the number of uterine implantations was the same in both the treated and control groups. The number and size of live fetuses were lower in the treated groups than in the control group. The data presented suggest that these effects may be dose-dependent, although the authors had not investigated the specific dose response relationship. Stoker *et al.* (1985) administered coal-tar (750 mg/kg) to female CD-1 mice by gavage on days 7 through 15 of gestation. Eighteen control and fifteen treated animals were used. Although the number of implantations was the same in the control and treated groups, the number of pups delivered was significantly lower in the treated group. The number of dams with complete litter resorption was higher in the treated group than in the control group. In addition, litter weights in the treated groups were lower than those in the control group.

Wolfe and Bryan (1939) treated rats with either B[a]P, benz[a]anthracene (B[a]A) or dibenz[a,h]anthracene (DB[a,h]A) by sc injections, daily from the first day of pregnancy. Doses of 5 mg/day resulted in fetal death and resorption for all three PAH (Wolfe and Bryan, 1939). Barbieri *et al.* (1986) administered used a single intraembryonic injection of B[a]P, benzo[a]pyrene, 6-methyl-, (BP6M) and two B[a]P metabolites, benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-(±) (BP45E) and benzo[a]pyrene, 7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (±) (BPDE-II) to developing Swiss mouse embryos. At the 0.1 mg dose, B[a]P and BP45E had no apparent effect on fetal development. Administration of BP6M at the same dose caused multiple malformations. The most potent compound tested was BPDE-II, a diol-epoxide metabolite of B[a]P. Diol-epoxides are

thought to be the reactive metabolites of PAH responsible for the biological effects of PAH (see appendix D). Intraembryonic injection of BPDE-II (0.1 mg) caused 85 percent fetal mortality and 100 percent fetal malformations (Barbieri *et al.* 1986). The mechanism by which BP6M exerts its effects is not understood, but some metabolic studies have suggested that cytochrome P-450c oxidation of the methyl group may generate a radical which is capable of binding to DNA (see appendix D). Shum *et al.* (1979) reported that ip administration of 200 mg/kg B[a]P (5.0 mg if a weight of 25 g/mouse is assumed) to C57BL/6N (responsive) and AKR/J (non-responsive) mice on day 7, 10 or 12 of gestation resulted in a greater degree of stillbirths and malformations in the responsive strain than in the non-responsive strain.

The terms "*responsive*" and "*non-responsive*" refer to the level of aryl hydrocarbon hydroxylase (AHH) induction that can be achieved in response to treatment with known inducers of AHH activity. Animals that are responsive show a significant increase in cytochrome P-450c-associated AHH activity, i.e. to AHH induction, when compared to control animals. Non-responsive animals show only marginal increases in AHH activity. Aryl hydrocarbon hydroxylase is the enzyme primarily responsible for the oxidative metabolism of PAH and the formation of diol-epoxides (see appendix D). Induction of AHH activity is mediated by the Ah receptor mechanism, which also controls a number of other responses including body weight loss and thymic atrophy (Poland, 1984). Many PAH can act as inducers of AHH activity. Therefore, animals which are responsive to AHH induction could be expected to show a greater sensitivity to diol-epoxide mediated effects than non-responsive animals. Barbieri *et al.* (1986) showed that the diol-epoxide derivatives of B[a]P are potent inducers of fetal malformations and mortality. Shum *et al.* (1979) suggested that responsiveness to AHH induction also has an effect on fetal development. Together, these reports indicate that the diol-epoxides play a significant role in fetal malformations and mortality. Further, the genetic predisposition to AHH induction could play an important role in determining the degree to which an individual's reproductive system will be affected by PAH.

The effects of responsiveness on fetal development was further investigated by Shum *et al.* (1979). By crossbreeding responsive and non-responsive mice, Shum *et al.* were able to show that responsive fetuses developing in non-responsive dams had greater levels of malformations, resorptions and stillbirths than non-responsive fetuses in the same litter. The same effects were not observed in both responsive and non-responsive fetuses of responsive dams. In responsive dams, both responsive and non-responsive fetuses showed the same levels of effects (Shum *et al.* 1979). The observed response is presumably due to the transplacental transfer of maternal metabolites to both types of fetuses, implicating that the genotype of both parents may determine the reproductive effects of PAH on the offspring.

Direct intraembryonic injection of BPDE-II required a much lower dose (0.1 mg) to induce fetal mortality than any of the other compounds examined (Barbieri, 1986). This finding suggests that BPDE-II may be a potent inducer of reproductive effects, and that fetal exposure to maternally

produced BPDE-II may play an important role in producing many of the effects observed. Barbieri's finding is consistent with the results of the fetal effect study conducted by Shum *et al.* (1979) in cross-bred mice. Non-responsive fetuses of non-responsive dams (where BPDE-II levels could reasonably be expected to be low) showed a lower level of fetal developmental anomalies than their responsive litter mates. However, non-responsive fetuses of responsive dams (where BPDE-II levels could be expected to be higher than those found in non-responsive dams) showed the same levels of fetal abnormalities as their responsive litter mates.

The data presented above suggest that exposure to either pure PAH or PAH containing mixtures in the diet (Rigdon and Rennels, 1963), by gavage (MacKenzie and Angevine, 1981; Khan *et al.* 1987; Stoker *et al.* 1985) or by injection (Wolfe and Bryan, 1939; Barbieri *et al.* 1986; Shum *et al.* 1979) can affect fetal development and viability, but does not seem to affect implantation. However, the doses required to produce effects are generally quite large, ranging between 5 mg/rat/day (20 mg/kg/day if a weight of 250 g/rat is assumed; Wolfe and Bryan, 1939) and 200 mg/kg (Shum *et al.* 1979) for pure PAH, and 750 mg/kg for PAH containing mixtures (Stoker *et al.* 1985). It is not possible to calculate accurate PAH doses for some of the studies cited (Rigdon and Rennels, 1963; Khan *et al.* 1987), although the effects reported were similar to those reported in other studies (MacKenzie and Angevine, 1981; Wolfe and Bryan, 1939; Barbieri *et al.* 1986; Shum *et al.* 1979). It is difficult to evaluate the effect of the exposure route on the observed effects. The reports cited had administered the test compound via a variety of exposure routes and the observed effects were similar in most cases. It should be noted, however, that in the absence of direct fetal administration, exposure to the parent compound or a metabolite can only happen through placental transfer. Therefore, if the routes used to administer the compound to the parent do not affect the maternal metabolism of the test compound, it is unlikely that route difference will be noted in the fetus. If, however, the route of administration has an effect on maternal processing, then route-dependent effects could be anticipated in the fetus. At present, there are insufficient data available to evaluate any possible route-specific effect on fetal mortality.

2.7.2 Fetal Tumorigenicity

Animals exposed to PAH *in utero* have been shown to develop tumor (Tomatis *et al.* 1971; York *et al.* 1984; Napalkov and Alexandrov, 1974; Rice *et al.* 1978; Urso and Gengozian, 1982, 1984). Data are available for 3-MC (Tomatis *et al.* 1971; York *et al.* 1984), DMBA (Napalkov and Alexandrov, 1974; Rice *et al.* 1978) and B[a]P (Urso and Gengozian, 1982, 1984) and are discussed below.

Tomatis *et al.* (1971) treated pregnant CF-1 mice with 3-MC starting 7 days before delivery. The dams were given three treatments of 2.8 mg of 3-MC in 0.2 ml olive oil by gavage, for a total dose of 8.4 mg. The treatments were spaced 24 hours apart. Within 20 weeks postpartum, all of the offspring exposed to 3-MC *in utero* were found to have tumors. The tumor incidence in the control

group was approximately 40 percent for the same period. The group sizes for the treated and control animals were 98 and 89, respectively. The data show that *in utero* exposure to 3-MC can significantly increase the incidence of tumors.

York *et al.* (1984) studied lung tumorigenesis in the responsive (AHH inducible) and non-responsive (AHH non-inducible) offspring from DBA/2J (non-responsive) dams bred to F1 (C57BL/6N X DBA/2J) males. Dams were treated daily on days 15, 16 and 17 of gestation with 3-MC at 7, 21 or 63 mg/kg/day. The lungs were found to be the major site of tumors in the offspring 6 months postpartum. Dose-related tumorigenic responses were noted in both responsive and non-responsive progeny. However, the non-responsive young had a higher level of tumor incidence than their responsive litter mates. In addition, the difference in the levels of the response between the two groups increased with dose. At the low treatment dose (7 mg/kg/day), 40 percent of the non-responsive offspring (6 of 15 animals) were found to have lung adenomas, while none of the responsive animals were found to have adenomas. At the intermediate dose (21 mg/kg/day), 70 percent of the non-responsive (9 of 13 animals) developed adenomas as compared to 40 percent in the responsive animals. At the high treatment dose (63 mg/kg/day), 82 percent of the non-responsive mice (22 of 27 animals) were found to have adenomas as compared to 43 percent in the responsive strain (3 of 7 animals).

Napolkov and Alexandrov (1974) showed that the offspring of random bred albino rats treated with a single iv dose of DMBA (15 mg/kg) on day 21 of gestation showed a higher incidence of tumors than the young from the control group. Similar results were observed by Rice *et al.* (1978) when pregnant SD rats were treated with a single ip injection of DMBA (24 mg/kg) on day 20 of gestation.

Urso and Gengozian (1982, 1984) examined the effect of *in utero* exposure to B[a]P on tumor development in both the male and female offspring of C3H/Anf mice. Pregnant C3H/Anf mice were treated either during mid-gestation (days 11 to 13) or late gestation (days 16 to 18) with an ip administration of B[a]P (150 mg/kg) (Urso and Gengozian, 1982). Although not specifically stated, the data presented suggest that only a single dose had been used. Calculating a total dose is only possible, however, if a single treatment is assumed. The liver was the primary tumor site in the male and ovarian tumors were predominant in the female 18 to 24 months postpartum. In addition, the authors demonstrated that the incidence of lung tumors increased following late gestational treatment compared to mid-gestational treatment. In a subsequent study (Urso and Gengozian, 1984), using the same dose of B[a]P and strain of mouse but a different treatment time (between days 11 and 17 of gestation), Urso and Gengozian showed that the tumor incidence in the animals exposed to B[a]P was 8 to 10 fold greater than that seen in the control group . As with the previous study, it is unclear if a single dose or repeated doses had been used.

The data summarized above indicate that *in utero* exposure to 3-MC, DMBA or B[a]P can result in an increased incidence of tumors in both mice and rats. As with fetal mortality, it is difficult to evaluate the effect of the route of administration on tumorigenic response, except to say that the various routes used (gavage, iv and ip injections) produce similar effects in the same dose range. In the absence of direct fetal administration, exposure to the parent compound or a metabolite can only happen through placental transfer. Therefore, if the routes used to administer the compound to the parent do not have an effect on the maternal metabolism of the test compound, it is unlikely that route difference will be noted in the fetus. If, however, the route of administration has an effect on maternal processing, then it is possible that route-dependent effects could be seen in the fetus. At present, the available data are insufficient to evaluate this possibility.

2.7.3 Fetal Immunosuppression

Only limited available data examine the effect of *in utero* exposure to PAH on the immune system. Urso and Gengozian (1982, 1984) monitored the effects of *in utero* exposure to B[a]P on the humoral and cell-mediated immune responses in C3H/Anf mice. Brief descriptions of the humoral and cell-mediated immune responses can be found in section 2.8 Immunotoxicity.

Urso and Gengozian (1982) treated pregnant C3H/Anf mice with B[a]P (150 mg/kg) by ip injection during mid-gestation (days 11 to 13) and late gestation (days 16 to 18). While not specifically stated, the data suggest that a single treatment was used. It does seem certain that the final dose was either 150 mg/kg or 450 mg/kg (3 x 150), although the actual dose used cannot be determined. Mid-gestational exposure to B[a]P caused a 75 percent reduction in the humoral immune response of plaque-forming cells to sheep red blood cells (SRBC) in the offspring at one week of age. At four weeks of age, there was an apparent return to control SRBC response levels. This recovery was, however, transient. By 78 weeks of age, the SRBC response in the treated animals was only 30 percent of the control. Exposure during late gestation produced a similar effect, but the level of immunosuppression in these animals was lower than that seen in the animals treated in mid-gestation, although the difference does not appear to be statistically significant.

Urso and Gengozian (1984) treated pregnant C3H/Anf mice with B[a]P (150 mg/kg) by ip injection on days 11 to 17 of gestation. As before, it is difficult to determine whether a single dose or if repeated doses had been used. The results showed that the cell-mediated immune response was significantly lower in the animals exposed to B[a]P *in utero*. Furthermore, this suppression appeared to persist for 78 weeks.

While the data presented above are limited to only two B[a]P studies in a single mouse strain, *in utero* exposure to PAH appears to have the potential to cause substantial and persistent harmful effects on the immune system. It should be noted, however, that the route of administration (ip) used in the cited studies is artificial and does not represent a plausible environmental pathway. The effect, if any, that maternal metabolism may have on the observed effects cannot be addressed with the

present data. Furthermore, the dose used to produce the observed immunosuppressions are quite large and are unlikely to represent typical environmental exposures. Additional work using much lower doses is required before the relevance of these effects can be adequately determined.

2.7.4 Fetal Clastogenicity

Oral administration of B[a]P (150 mg/kg) to pregnant C57 (responsive) and DBA (non-responsive) mice on day 11 of gestation caused clastogenic aberrations in the progeny (Adler *et al* 1989). The clastogenic response was lowest in the embryos that were homozygous responsive (C57 X C57) and highest in the hybrid embryo (C57 X DBA) (Adler *et al.* 1989). The homozygous DBA embryo (DBA X DBA) showed a level of clastogenicity that was midway between that observed in the homozygous C57 and the hybrid (C57 X DBA) (Adler *et al.* 1989). The authors concluded from these data that the clastogenic response was determined, in part, by the rate of B[a]P metabolism in both the dam and the progeny (Adler *et al.* 1989). In addition, other genetically controlled processes, such as DNA repair and transformations of the primary DNA lesions to DNA strand breaks, may also play an important role in fetal clastogenic effect (Adler *et al.* 1989).

2.7.5 Fertility

Exposure to PAH has been shown to affect fertility in both male and female rats and mice. The data available for both males and females are discussed below.

2.7.5.1 Male

Payne *et al.* (1958) treated mature (six month old) male rats with a single ip injection of B[a]P (4 mg). Twelve months post treatment, a large portion of the treated animals showed atrophy of the seminiferrous tubules with the absence of spermatids and spermatozoa. Wyrobek and Bruce (1975) treated F1 (C57BL X C3H1) male mice with either B[a]P or 3-MC. The animals were treated by ip injection for 5 consecutive days. Two B[a]P doses were used: 20 and 100 mg/kg (doses are equivalent to 5 mg and 25 mg if a weight of 250 g/rat is assumed). The graphical data presented by the authors makes it difficult to determine accurately the 3-MC dosages used. The low dose is clearly less than 10 mg/kg and may be as low as 5 mg/kg. The high dose would appear to be approximately 50 mg/kg. At the low dose, B[a]P (20 mg/kg) did not produce an increase in sperm abnormalities above those found in the control ($\approx$ 2.5 %), 1, 4 and 10 weeks post treatment. At the high dose (100 mg/kg), B[a]P did not produce an increase in sperm abnormalities 1 week post treatment. At 4 and 10 weeks post treatment however, abnormalities increased to approximately 15 and 20 percent, respectively. Similar effects were observed with 3-MC. One week post treatment, no effects were noted at either the high or low doses. The low dose treatment also did not produce effects 4 or 10 weeks post treatment. At 4 weeks post

treatment, the high dose (≈ 50 mg/kg) of 3-MC increased the levels of sperm abnormalities to approximately 12 percent. By 10 weeks post treatment, however, the levels had returned to approximately 4 percent, marginally above the control. The authors do not provide an explanation for the observation.

As indicated in the data presented, some PAH (B[a]P and 3-MC) appear to be able to affect the tissue responsible for sperm production, resulting in decreased fertility and reduced breeding success. It is not possible to evaluate the effect that the route of administration may have, as the cited studies used the same method of treatment (ip injection). In both studies, the levels used (5 to 100 mg/kg) are relatively large and the route of administration (ip injection) is not a plausible environmental exposure route. Consequently, it is also difficult to determine if normal environmental exposures would likely lead to sperm abnormalities in humans.

2.7.5.2 Female

Mattison and Thorgeirsson (1979), and Shiromizu and Mattison (1982) reported that intraovarian (io) injection of B[a]P caused oocyte destruction. Shiromizu and Mattison (1982) administered B[a]P by io injection at doses of 1 μg and 10 μg per ovary to C57BL/6N and DBA/2J mice. Treatment at the low dose caused 30 percent and 40 percent oocyte destruction in DBA/2J and C57BL/6N mice, respectively. At the 10 μg/ovary dose, 55 and 78 percent oocyte destruction were found in the DBA/2J and C57BL/6N mice, respectively. Mattison and Thorgeirsson (1979) treated C57BL/6N and DBA/2J mice with a single io injection of B[a]P, 3-MC or DMBA (dose for all compounds was 80 mg/kg) six days prior to sacrifice. In the DBA/2J mouse strain, treatment with B[a]P, 3-MC and DMBA caused 12.5, 46.2 and 82.5 percent oocyte reduction per ovary, respectively. In the C57BL/6N strain, treatment with B[a]P, 3-MC or DMBA caused 65.5, 99.1 and 99.4 percent oocyte reduction, respectively. The data suggest that the responsive mouse strain (C57BL/6N) is more sensitive to the effects of PAH on oocyte destruction than the non-responsive (DBA/2J) strain. The levels of AHH activity in the ovary are greater in the C57BL/6N strain than in the DBA/2J strain (Mattison and Thorgeirsson, 1979). As AHH is responsible for the metabolism of PAH and the formation of diol-epoxides, the higher levels of ovarian AHH activity and oocyte destruction in the C57BL/6N as compared to the DBA/2J strain suggest that ovarian metabolism of PAH may play a role in oocyte destruction.

2.7.6 Summary

The data presented in the preceding sections show that exposure to PAH can have a number of effects on both the female and male reproductive systems, and fetal development. The largest amount of data pertain to fetal development, where reported effects include malformations, stillbirths, resorptions, immunosuppression, clastogenicity and tumorigenicity (see sections 2.7.1 through

2.7.4). The doses required to produce the fetal developmental effect are somewhat higher than those required to elicit a carcinogenic response (see appendix A). Although no human data are available, fetal developmental effect may be important in humans.

It is difficult to evaluate the effect that the route of administration has on the fetal or adult effects reported. The primary reason is that oral administration is the only real exposure route used. Injection studies do not truly represent what could be considered "normal" routes of exposure. Secondly, administration of a compound to a pregnant animal to produce effects in the fetus requires that placental transfer takes place. Thus in the absence of direct administration to the fetus, the fetus has only one route of exposure. This also raises the issue of maternal processing and placental transfer of maternally produced metabolites. As has been shown (Shum *et al.* 1979, section 2.7.1), maternal transfer of PAH metabolites can have a marked effect on the fetus. The data presented indicate that similar effects were noted in fetuses regardless of the route of treatment used for the mother and suggest that the route of administration may not be a concern. If the route of maternal administration alters maternal metabolism, then alterations in fetal effects may occur. However, the available data are insufficient to adequately evaluate this possibility.

2.8 Immunotoxicity

The effects of PAH on the immune system were first reported by Malmgren *et al.* (1952) who reported reduced hemolysin titers in mice treated with 3-methylcholanthrene (3-MC), dibenz[a,h]anthracene (DB[a,h]A) or benz[a]anthracene (B[a]A). Since this initial study, PAH have been shown to cause cell-mediated and humoral immune responses in experimental animals. The majority of the available studies administered PAH to experimental animals by either subcutaneous (sc) or intraperitoneal (ip) injection. A few studies have reported immune system effects following gavage or intratracheal instillation. The effects of PAH administered by injection, gavage or intratracheal instillation on the cell-mediated and humoral immune responses are discussed below. No data on the effects of PAH exposure on the human immune system could be located in the literature.

2.8.1 Cell-Mediated Immunity

Cell-mediated immunity (CMI) refers to immune responses for which the thymus-dependent (T-cells) are the principal effector cells (White, 1986). The primary function of the CMI response is the maintenance of homeostasis by protecting against various infectious agents (Weir, 1983). It may also play a role in the elimination of spontaneously arising neoplastic cells (Weir, 1983). A number of tests have been used to monitor the effects of xenobiotics, including PAH, on the CMI system. The most commonly reported test is lymphocyte proliferation in response to stimulation by phytohemagglutinin (PHA). Other tests include Natural Killer cell cytolysis and cytotoxic T-cell cytolysis. Reviews of CMI and the tests used to monitor CMI competency can be found in Weir

(1983), Dean *et al.* (1985) and White (1986). A number of authors (Wojdani *et al.* 1984a; Wojdani and Alfred, 1984b; Lubet *et al.* 1984; Dean *et al.* 1985; Lyte and Bick, 1985 and Johnson *et al.* 1990) have examined the effects of various PAH on the CMI response in experimental animals. The results from these studies are summarized and discussed below.

Wojdani *et al.* (1984a) monitored the effects of PAH exposure on the lymphocyte binding and killing rates in splenic lymphocytes (SL) and peritoneal exudate lymphocytes (PEL) in C57BL/6 and C3H mice. Animals were treated with a single ip injection of either B[a]P, B[e]P or 3-MC at four doses (0, 0.5, 5.0 and 50 mg/kg). Both 3-MC and B[a]P produced dose-dependent suppression of the killing and binding activity in both SL and PEL. At the low dose (0.5 mg/kg), 3-MC and B[a]P had no apparent effect on either killing or binding in either SL or PEL in both strains of mice. At the high dose (50 mg/kg), 3-MC reduced the killing activity of SL and PEL by 64 and 73 percent, respectively in the C57BL/6 mice and by 59 and 67 percent, respectively in the C3H mice. Antigen binding activity in SL and PEL were reduced by 42 and 39 percent, respectively in C57BL/6 mice and by 36 and 60 percent, respectively in C3H mice. At the highest dose, B[a]P reduced the killing activity of SL and PEL by 69 and 67 percent, respectively in C57BL/6 mice and by 66 and 54 percent, respectively in C3H mice. Antigen binding in both SL and PEL was reduced by 50 percent in C57BL/6 mice. In C3H mice, binding in SL was reduced by 43 percent and by 70 percent in PEL. Administration of B[e]P had no appreciable effect on binding or killing in either SL or PEL in both strains of mice. These data suggest that PAH which have been demonstrated to have carcinogenic properties (3-MC and B[a]P) also have a suppressive effect on CMI. Those PAH which have no significant carcinogenic activity (B[e]P) have no appreciable effect on the CMI response.

Wojdani and Alfred (1984b) examined the effects of PAH exposure on T-lymphocyte proliferation in response to stimulation with PHA in C57BL/6, C3H and DBA/2 mice. Twenty-four to 48 hours prior to sacrifice, animals were treated with a single ip injection of either, 3-MC, B[a]P or B[e]P at dosages of 0, 2,5, 10 and 50 mg/kg. A dose-dependent reduction in T-lymphocyte proliferation was reported in C57BL/6 and C3H mice. In C57BL/6 mice, the two highest doses (10 and 50 mg/kg) produce 40 and 63 percent reductions in lymphocyte proliferation, respectively. The same dose levels in C3H mice produced 21 and 57 percent reductions, respectively. In the DBA/2 strain, treatment with 3-MC did not have an effect on T-lymphocyte proliferation. Neither B[a]P nor B[e]P was reported to have a suppressive effect on T-lymphocyte proliferation in any of the mouse strains tested. The authors note that the ability of 3-MC to cause suppression of T-lymphocyte proliferation in both AHH responsive mouse strains (C57BL/6 and C3H) and not in the non-responsive DBA/2 strain may indicate that immunosuppression may play a role in the tumorigenicity of these compounds. However, the failure of the carcinogenic B[a]P to produce any immunosuppressive effects in this study was not addressed by the authors.

Lubet *et al.* (1984) studied the effect of 3-MC on T-lymphocyte proliferation in response to stimulation with PHA in responsive C57BL/6N and non-responsive DBA/2N mice. Six days prior to sacrifice, animals were given a single ip injection of 3-MC at doses of 0, 14, 42, 125 and 375 mg/kg. In the responsive C57BL/6N strain, a dose-dependent suppression of T-lymphocyte proliferation was reported. In the non-responsive DBA/2N strain, suppression was reported only at the highest dose (375 mg/kg). At the highest dose, C57BL/6N and DBA/2N mice showed 96 and 60 percent reductions in T-lymphocyte proliferation, respectively. The difference in the levels of immunosuppression between the responsive and non-responsive strains suggests that the Ah receptor may play a role in modulating the immunosuppressive effects of PAH.

Dean *et al.* (1985) monitored the effect of DMBA on PHA-induced T-lymphocyte proliferation in female F1 (C57BL/6 X C3H) (B6C3F1) mice. Animals were treated with 10 sc injections over a two week period yielding total administered doses of 5, 50 and 100 mg/kg. All three doses produced significant suppression of T-lymphocyte proliferation. However, there does not appear to be a dose-dependent suppression over the dose range tested. The three doses produced 45, 48 and 43 percent reductions, respectively (Dean *et al.* 1985). The data are insufficient to determine if this reduction represents a maximum level of suppression. At a dose of 50 mg/kg 3-MC produced a similar level of suppression (63%) (Wojdani and Alfred, 1984b) in C57BL/6 mice, and at a dose of 125 mg/kg, it produced a 59 percent reduction (Lubet *et al.* 1984). Further suppression with 3-MC was only reported at a three-fold higher dose (375 mg/kg) where a 96 percent reduction in T-lymphocyte proliferation was reported (Lubet *et al.* 1984). Therefore, it is possible that higher doses of DMBA would produce greater levels of suppression.

Lyte *et al.* (1987) reported that sc administration of B[a]P to female B6C3F1 mice suppressed the production of interleukin-2 in splenocytes. Animals were treated with daily sc injections of B[a]P (5, 20 and 40 mg/kg) for 14 days and were sacrificed on day 15. At the high dose (40 mg/kg) B[a]P produced a 62 to 78 percent inhibition of interleukin-2 production. Production of interleukin-3 did not appear to be affected. Interleukin-2 and -3 are required for the maturation of both T and B cells (Lyte *et al.* 1987). Therefore, inhibition of interleukin-2 production could lead to immunosuppression by reducing the number of T-cells from further developing.

Johnson *et al* (1990) examined the effect of 3-MC exposure on cell-mediated cytokine activity in C57BL/6J and DBA/2J mice. The animals were given 3-MC (40 or 80 mg/kg) by sc injection. Treatment with 3-MC at 40 mg/kg caused a 17 percent reduction in cytokine activity in DBA/2J mice which was not considered to be statistically different from control. At 40 mg/kg, 3-MC produced a 29 percent reduction in cytokine activity in the C57BL/6J strain, which was considered to be statistically significant. At the high dose, (80 mg/kg), 33 and 43 percent reductions in cytokine activities were reported for the DBA/2J and C57BL/6J strains, respectively. Both of these differed statistically from the control.

The data cited above clearly indicate that some PAH are able to affect various components of the CMI response. A number of authors have shown that 3-MC (Wojdani and Alfred, 1984b; Lubet *et al.* 1984) and DMBA (Dean *et al.* 1985) can produce dose-dependent reductions in the lymphocyte proliferation response to PHA. Other PAH (e.g. B[a]P and B[e]P), do not appear to have a significant effect on the lymphocyte proliferation response to PHA stimulation (Wojdani and Alfred, 1984b). Benzo[a]pyrene has been shown to have dose-dependent effects on other CMI responses including binding and killing activities of splenic and peritoneal exudate lymphocyte (Wojdani *et al.* 1984a), and suppression of interleukin-2 production (Lyte *et al.* 1987). Suppression of SL and PEL binding or killing (Wojdani *et al.* 1984a) and reduction in cytokine activity have also been reported following exposure to 3-MC. Exposure to B[e]P does not appear to have any effects on the CMI response. The data would suggest that the immunosuppressive effect of PAH are restricted to those PAH which have been shown to have carcinogenic activity in the mouse (see appendix A). Furthermore, it would appear that the immunosuppressive effects are dependent on the individual PAH, as different compounds seem to elicit different responses. There are, unfortunately, insufficient data available to properly evaluate this observation. Also, as all the cited studies administered the test compounds by either sc or ip injection, it is not possible to evaluate the effect of the route of administration on the reported responses.

2.8.2 Humoral Immunity

Humoral immunity (HI) encompasses immune responses where B cells (lymphocytes derived directly from the bone-marrow) are the primary effector cells (White, 1986). This type of immune response is characterized by the appearance of immunoglobulins (antibodies) in the blood. Immunoglobulins are capable of binding to the antigen that stimulate their production (Weir, 1983). The result of this binding may be detoxification of the antigen. Binding may also result in a clumping of the antibody/antigen complex (plaque formation) that is subsequently destroyed by phagocytic cells (Weir, 1983). Experimentally, effects on the HI system are determined by measuring the number of plaque forming cells (PFC) produced in response to an antigen challenge (Dean *et al.* 1985; White, 1986). The most commonly used antigen appears to be sheep red blood cells (SRBC). A more complete discussion of the HI response and the tests used to monitor HI competence can be found in Weir (1983), Dean *et al.* (1985) and White (1986).

A number of authors (Malmgren *et al.* 1952; Stjernswärd, 1966; Levy *et al.* 1977; Lubet *et al.* 1984; Dean *et al.* 1985; Lyte and Bick, 1985; White *et al.* 1985; Schnizlein *et al.* 1987) have examined the effects of a number of PAH on the HI response in experimental animals. The results from these studies are discussed briefly below.

Malmgren *et al.* (1952) were the first to report the effects of PAH on the immune system. Mice (BalB/C strain) were treated with 3-MC (25, 50 and 100 mg/kg), DB[a,h]A (50, 100 and 400 mg/kg), B[a]A (100 and 400 mg/kg) or phenanthrene (phen) (800 and 1600 mg/kg) by sc injection for three consecutive days. The effects of PAH exposure on the hemolysis response to SRBC was monitored. The results showed that 3-MC produced suppression of the hemolysis response at the 50 and 100 mg/kg dose levels. Both DB[a,h]A and B[a]A produced suppression but only at the high dose (400 mg/kg). Treatment with phen failed to produce a response even at the high dose level of 1600 mg/kg. The authors indicated that the immunosuppressive effect parallelled the reported carcinogenicity of these compounds (Malmgren *et al.* 1952). The carcinogenic potency of 3-MC is greater than that of the other compounds tested (see appendices A and C) and produced greater effects on hemolysis at lower doses than the other compounds. However, there is a large difference between the carcinogenic potencies of DB[a,h]A and B[a]A (see appendices A and C), and this report shows a similar level of immunosuppression for these two compounds. It should be noted that Malmgren *et al.* (1952) reported effects as a subjective grading of effects, so the discrepancy between reported immunosuppressive effects and calculated carcinogenic potencies may be a reflection of the subjective nature of the system used by Malmgren *et al.* (1952).

Stjernswärd (1986) examined the immunosuppressive effects of several PAH in CBA mice. The mice were treated with intramuscular (im) injections of anthracene (anth), B[e]P, B[a]P, 3-MC or DMBA (1 mg/animal) and were subsequently immunized with SRBC 2, 7 or 32 days after treatment with the test compound (Stjernswärd, 1966). No suppression of PFC function towards SRBC was seen in the animals treated with either anth or B[e]P. In animals immunized on days 2, 7 or 32, treatment with B[a]P produced 66, 43 and 1 percent suppression of the PFC response to SRBC, respectively. In animals treated with 3-MC, the levels of suppression were 72, 70 and 17 percent in animals immunized on days 2, 7 or 32. Treatment with DMBA produced 56, 43 and 74 percent suppression of the PFC-SRBC response respectively (Stjernswärd, 1966). The data suggest that the immunosuppressive effects of DMBA are more persistent than those of either B[a]P or 3-MC. Studies covering a longer time span would be useful in determining if the immunosuppressive effects of DMBA persist over a significantly longer time period than those of the other PAH tested.

Levy *et al.* (1977) treated C57BL/6 and DBA/2 mice with intratracheal installations of 3-MC (500 μg). The animals were immunized with goat erythrocytes (GRBC) six and 16 days post treatment. In C57BL/6 mice (responsive strain), 51, 53 and 36 percent suppression of the IgG-PFC responses were reported 19, 23 and 25 days post treatment with 3-MC (3, 7 and 9 days after last immunization with GRBC) (Levy *et al.* 1977). The non-responsive DBA/2 mice showed no significant reduction in the IgG-PFC response to GRBC (Levy *et al.* 1977). The data show that, in mice, the immunosuppressive effects of PAH seem to be strain dependent. In addition, this dependence appears to be associated with the *Ah*-responsiveness of the animals. It would suggest that animals more sensitive to the carcinogenic effects of PAH may also be more susceptible to the immunosuppressive effects. However, more data are required to support this hypothesis.

Lubet *et al.* (1984) monitored the PFC-response to SRBC in C57BL/6N and DBA/2N mice following ip treatment with 3-MC. Animals were given a single ip injection of 3-MC (5, 25 or 125 mg/kg) 5 days prior to a single immunization with SRBC (Lubet *et al.* 1984). A dose-dependent decrease in the PFC response to SRBC was reported in both mouse strains. At the highest dose, 93 and 81 percent suppression was observed in the C57BL/6N and DBA/2N strains, respectively. At the 25 mg/kg dose, a substantial difference in the levels of suppression was noted between the two strains. In the C57BL/6N strain, 25 mg/kg 3-MC produced an 80 percent suppression of the PFC response, while only an 11 percent suppression was reported in the DBA/2N strain (Lubet *et al.*, 1984). Lubet *et al.* also reported the effects of DB[a,h]A and benzo[b]triphenylene (BbTrip) [Lubet *et al.* identified this latter compound as dibenz[a,c]anthracene] on the PFC-response in both strains of mice. Treatment with DB[a,h]A (single ip injection) at 25, 50 and 100 mg/kg produced 77, 87 and 89 percent suppression of the PFC response to SRBC in C57BL/6N mice and 6, 50 and 94 percent suppression in DBA/2N mice (Lubet *et al.* 1984). Treatment with BbTrip at 25, 50 and 100 mg/kg produced 9, 0.5 and 13 percent suppression of the PFC-SRBC response in C57BL/6N mice and 13, 19 and 26 percent suppression in the DBA/2N strain (Lubet *et al.* 1984). At the high doses (125 mg/kg and 100 mg/kg), the levels of immunosuppression observed for 3-MC and DB[a,h]A did not appear to differ much in the two strains of mice. At the lower doses, however, the C57BL/6N strain appeared to be more sensitive to the immunosuppression than the DBA/2N strain. For BbTrip, the results appeared to be reversed. The levels of immunosuppression in the DBA/2N strain was greater than that in the C57BL/6N strain. In both mouse strains, the levels of immunosuppression are relatively low and may not be statistically different from the control. More information is required before these data can be interpreted.

Oral administration of 3-MC (11, 44 and 100 mg/kg/day) for four consecutive days to C57BL/6N and DBA/2N mice produced immunosuppression in the DBA/2N strain but not in the C57BL/6N strain (Lubet *et al.* 1984). At 44 and 100 mg/kg/day, the DBA/2N strain showed 13 and 65 percent suppression, respectively. No significant suppression was reported in the C57BL/6N strain at the same doses (Lubet *et al.* 1984). A greater level of depression of the immune response in the DBA/2N strain than in the C57BL/6N strain following oral administration is the reverse of the pattern seen when the compounds are administered via ip injection. This difference is difficult to explain, but may be due in part to the greater capacity of the C57BL/6N strain to metabolize and remove PAH than the DBA/2N strain. Thus, orally administered PAH would be more effectively removed by hepatic metabolism in the C57BL/6N strain than in the DBA/2N strain, thereby leaving an effectively lower PAH concentration available to effect the immune system than might be found in the DBA/2N strain. In addition, lymphoid tissue has been shown to be inducible (Lubet *et al.* 1984). Therefore, the greater level of PAH parent compound which may be available to the lymphoid tissue in the DBA/2N strain may result in the formation of a greater level of reactive metabolites in the lymphoid tissue of the DBA/2N strain than would be found in the C57BL/6N strain. The result may be an increased immune response in the DBA/2N strain. Administration of the PAH by injection

may mean that the levels of parent compound available to the lymphoid tissue is the same in both strains. Therefore the greater capacity of the C57BL/6N strain to metabolize PAH would result in a greater level of response. It should be noted that this explanation is largely conjecture and, as yet, data to support or refute it are not available.

Dean *et al.* (1985) treated female B6C3F1 with sc injections of DMBA (5, 50 and 100 mg/kg total dose) in multiple treatments over a 10 day period. The immunoglobulin M (IgM) response to SRBC showed a dose-dependent suppression that ranged between 25 percent at the low dose and 97 percent at the high dose.

White *et al.* (1985) examined the immunosuppressive effects of a number of PAH including perylene (pery), anth, chrysene (chry), B[e]P, B[a]A, BbTrip, DB[a,h]A, DMBA, B[a]P and 3-MC in B6C3F1 and DBA/2 mice. In B6C3F1 mice, daily sc treatment with pery (40 mg/kg/day), anth (29 mg/kg/day), chry (37 mg/kg/day) or B[e]P (40 mg/kg/day) for 14 days (equivalent to 160 µmoles/kg/day for all PAH) produced no effect on the IgM-PFC response to SRBC. Similar treatment with B[a]A, BbTrip, B[a]P and DB[a,h]A at 160 µmoles/kg/day (37 mg/kg/day, 45 mg/kg/day, 40 mg/kg/day & 45 mg/kg/day, respectively) produced 56, 55, 64 and 91 percent reductions in the IgM-PFC response to SRBC, respectively. Treatment with DMBA or 3-MC at 20 µmoles/kg/day (43 mg/kg/day and 41 mg/kg/day, respectively) produced 76 and 23 percent reduction, respectively. In DBA/2 mice, treatment with B[e]P and B[a]P (160 µmoles/kg/day) by sc injection for 14 days produced no immunosuppression for B[e]P and 93 percent suppression with B[a]P. Treatment with DMBA or 3-MC at 20 µmoles/kg/day produced 93 and 49 percent suppression of the IgM-PFC response to SRBC. These data suggest that DBA/2 mice are more sensitive to the immunosuppressive effects of PAH when administered via sc injection. This observation is contrary to the responses reported by Levy *et al.* (1977) and Lubet *et al.* (1984) who showed that C57BL/6 mice were more sensitive to the immunosuppressive effects of PAH than the DBA/2 strain when the compounds were administered by ip injection. This finding suggests that the route of administration may play an important role in the suppression of the HI response. Further data are required to adequately evaluate the effect of the route of administration on immunosuppression by PAH.

Lyte and Bick (1985) studied the effect of age on the immunosuppressive action of B[a]P. Three groups of B6C3F1 mice aged 3-6 months, 16-18 months and 23-26 months were treated with B[a]P (40 mg/kg) 4 days prior to and 4 days after immunization with SRBC. An age-dependent reduction in the PFC response to SRBC was reported. The mice in the 3-6 month age group showed 33 percent suppression, while the animals in the 16-18 month and 23-26 month age groups showed 74 and 93 percent suppression respectively.

Schnizlein *et al.* (1987) used intratracheal administration of B[a]P (0.4, 4.0 and 40 mg/kg) to monitor the effects on the lung associated lymph node (LALN) lymphocyte PFC response to SRBC. At the highest dose, a 60 percent suppression of the PFC response was reported. No significant effects were noted at any of the lower doses.

A number of reports (Stjernswärd, 1966; White *et al.* 1985; White, 1986; Lyte and Bick, 1985; Johnson *et al.* 1990; Wojdani and Alfred, 1984b; Malmgren *et al.* 1952) have suggested that the immunosuppressive effects of PAH may play a role in the carcinogenic activity of these compounds. The data cited above indicate that those PAH which show carcinogenic activity (B[a]P, 3-MC, DMBA, DB[a,h]A) also have a suppressive effect on the humoral immune system. The non-carcinogenic PAH for which data are available (B[e]P, anth, chry, pery, phen, BbTrip) have little or no effect on the HI in either the responsive (C57BL/6) or non-responsive (DBA/2) mouse strains. Studies that have used responsive and non-responsive strains of mice have shown that, in many cases, the *Ah*-responsive mice are more sensitive to HI immunosuppression than the non-responsive strains (White *et al.* 1985; Lubet *et al.* 1984; Levy *et al.* 1977). However, Lubet *et al.* (1984) and White *et al.* (1985) also demonstrated that the route of treatment plays a role in determining immunosensitivity. Lubet *et al.* (1984) showed that ip administration of PAH produced a greater immunosuppression in C57BL/6 mice than in DBA/2 mice, while oral administration had the opposite effect. The reasons for this difference are unclear, but may reflect differences in metabolism between the two strains. A number of studies which have examined the route-dependence of HI immuno-suppression have indicated that the route of administration may play a role in the expression of immunosuppression (Lubet *et al.* 1984; White *et al.* 1985). There is, however, insufficient information to adequately evaluate this observation.

2.8.3 Correlation Between Immunotoxicity and Carcinogenicity

A number of authors have suggested that a link may exist between the immunosuppressive effects and the carcinogenic activity of PAH (Stjernswärd, 1966; White *et al.* 1985; White, 1986; Lyte and Bick, 1985; Johnson *et al.* 1990; Wojdani and Alfred, 1984b; Malmgren *et al.* 1952). This suggestion is based on a number of factors. Firstly, one of the functions of the immune system is to recognize and remove items that are considered *non-self*, including spontaneously arising neoplastic cells (Weir, 1983). Secondly, suppression of this activity may, in part, be responsible for the development of tumors by hampering the identification and removal of neoplastic cells (Weir, 1983). Thirdly, a number of PAH which have been shown to have carcinogenic activity (DMBA, 3-MC, B[a]P & DB[a,h]A) have been shown to have immunosuppressive effects on both the humoral and cell-mediated immune systems. Therefore, a correlation may exist between the carcinogenic action of PAH and their ability to suppress the immune system.

Based on a review of currently available literature, White (1986) suggested that there is a poor correlation between carcinogenicity and immunosuppression for many parts of the immune system. As an example, White (1986) cited data which showed that DMBA suppressed many components of the CMI response, while B[a]P affected a very limited number of them. This finding agrees with the data presented by Wojdani *et al.* (1984a), who showed that 3-MC was able to suppress T-lymphocyte proliferation in C57BL/6 and C3H mice, while B[a]P and B[e]P both showed no effect

(see section 2.8.1). If carcinogenicity correlated with suppression of all components of the immune system, then B[a]P would be expected to generate some level of T-lymphocyte suppression. White (1986) suggested that a more consistent relationship could exist between the carcinogenic activity and the suppression of the HI response. The studies (cited in the previous section) which examined the effect of PAH on the HI PFC response to SRBC show that non-carcinogenic PAH have little or no effect on the PFC response to SRBC (see section 2.8.2). White *et al.* (1985) proposed a rank order for the immunotoxic potency of PAH.

DMBA > 3-MC > DB[a,h]A > B[a]P > B[a]A ≥ BbTrip > Chry > B[e]P

This order agrees favorably with the rank order of carcinogenic potencies of these compounds in rodents (see section 3.5.7).

DMBA > 3-MC > DB[a,h]A > B[a]P > Chry > B[e]P

In CD-1 mice, however, the agreement between immunotoxicity and carcinogenicity rank orders is not as solid (see section 3.5.7).

3-MC > B[a]P ≥ DMBA ≥ DB[a,h]A > Chry > B[e]P

While the CD-1 carcinogenicity rank order does not agree exactly with the immunosuppression order suggested by White *et al.* (1985), all immunosuppressive PAH show a greater level of carcinogenicity than those that do not show immunosuppressive behavior.

Although it is clear that a direct correlation may not exist between the immunosuppressive and carcinogenic activities of PAH, the data would suggest that the effects are related in some fashion. It could be that suppression of the body's ability to recognize and remove developing neoplastic cells by PAH enhances the carcinogenic effects by increasing the survival rate of cells that have become neoplastic as a result of PAH activity. It is also possible that the difference in carcinogenicity may be a function of both the compound's ability to produce transformed cells and its ability to suppress the immune response responsible for the removal of these transformed cells. One factor which must also be considered is the dosages at which these effects manifest themselves. The studies of immunosuppression cited have all used moderately large doses. The lowest dose that produced an effect in either the CMI or HI was 5.0 mg/kg for DMBA (Dean *et al.* 1985) which suppressed T-lymphocyte proliferation (see section 2.8.1). The doses at which the immunosuppressive effects of PAH have been reported are similar to those which elicit carcinogenic responses.

2.9 Carcinogenicity

The carcinogenicity of individual PAH and PAH-containing mixtures have been well studied in experimental animals (IARC, 1987; USDHHS, 1991). Virtually no data exists on the carcinogenicity of individual PAH in humans, and only a limited amount of data on the carcinogenicity of PAH containing mixtures is available for humans (IARC, 1987; USDHHS, 1991). There is evidence that a number of individual PAH are carcinogenic in experimental animals, while others have been found to be non-carcinogenic. For other PAH, the data are inadequate to determine whether the compounds are carcinogenic or not. Because of the varying carcinogenic activities of individual PAH, it is not possible to provide a single weight of evidence carcinogenicity assessment for PAH as a class. The carcinogenicity of each PAH must be assessed separately. Table 2.9.1 shows a list of PAH for which weight of evidence carcinogenicity assessments are available. While it is not possible to provide a carcinogenicity assessment for PAH as a class, it may be possible to evaluate the carcinogenicity of PAH-containing complex mixtures. There is good evidence that some PAH-containing complex mixtures are carcinogenic to humans. A list of PAH-containing complex mixtures for which weight of evidence carcinogenicity assessments are available are shown in table 2.9.2.

The assessment of PAH carcinogenicity is further complicated by the dependence of tumor type and location on the species and the route of administration. Administration of 3-MC to the respiratory tract in mice produces primarily pulmonary adenomas. Squamous-cell carcinomas have also been reported, but only at relatively high doses (3 mg 3-MC/mouse) (Nettesheim *et al.* 1971). In rats, treatment with 3-MC (3 mg/rat) produce squamous cell carcinomas while adenomas are virtually absent (Laskin *et al.* 1970). Topical application of DMBA, B[a]P or 3-MC tends to produce squamous-cell carcinomas in mice and rats (Bernland and Homburger, 1983). In hamsters, topical application of DMBA produces melanomas rather than squamous-cell carcinomas, while B[a]P and 3-MC do not appear to produce tumors (Bernland and Homburger, 1983). For some carcinogenic PAH, it has been shown that the route of administration has an effect on the type of tumor formed and its location (see sections 3.4.7 and 3.4.8). Administration of PAH to either the respiratory tract or the skin tends to form tumors at the site of initial contact. Oral exposure to PAH tends to produce tumors at sites removed from the initial point of contact (see section 3.4.7 and 3.4.8). Thus not only must the carcinogenic action be evaluated, but the route of administration and the species studied must also be considered when assessing the carcinogenicity of each PAH. The dose-response assessment of the scientific criteria document focuses on these issues and discusses each of them in detail.

Table 2.9.1 Weight of evidence carcinogenicity assessments for individual PAH.

Name	Carcinogenicity Assessment	
	IARC[1]	USEPA[2]
Acenaphthene, 5-nitro-,	2B	
Acenaphthylene		D
Anthanthrene	3	
Anthracene	3	D
Anthracene, 9-nitro-,	3	
Benz[a]acridine	3	
Benz[a]anthracene	2A	B2
Benzo[a]pyrene	2A	B2
Benzo[b]fluoranthene	2B	B2
Benzo[c]phenanthrene	3	
Benzo[e]pyrene	3	
Benzo[ghi]fluoranthene	3	
Benzo[ghi]perylene		D
Benzo[j]fluoranthene	2B	
Benzo[k]fluoranthene	2B	B2
Benzo[a]fluorene	3	
Benzo[b]fluorene	3	
Benzo[c]fluorene	3	
Carbazole	3	
Chrysene, 1-methyl-,	3	
Chrysene, 2-methyl-,	3	
Chrysene, 3-methyl-,	3	
Chrysene, 4-methyl-,	3	
Chrysene, 5-methyl-,	2B	
Chrysene, 6-methyl-,	3	
Chrysene, 6-nitro-,	3	
Chrysene	3	B2
Coronene	3	
Cyclopenta[cd]pyrene	3	
Dibenz[a,h]acridine	2B	
Dibenz[a,j]acridine	2B	
Dibenz[a,c]anthracene	3	
Dibenz[a,h]anthracene	2A	B2
Dibenz[a,j]anthracene	3	
7*H*-dibenzo[c,g]carbazole	2B	
Dibenzo[a,e]fluoranthene	3	

Table 2.9.1 Weight of evidence carcinogenicity assessments for individual PAH.....continued.

Name	Carcinogenicity Assessment	
	IARC[1]	USEPA[2]
Dibenzo[h,rst]pentaphene	3	
Dibenzo[a,e]pyrene	2B	
Dibenzo[a,h]pyrene	2B	
Dibenzo[a,i]pyrene	2B	
Dibenzo[a,l]pyrene	2B	
Fluoranthene	3	D
Fluoranthene, 2-methyl-,	3	
Fluoranthene, 3-methyl-,	3	
Fluoranthene, 3-nitro-,	3	
Fluorene	3	D
Indeno[1,2,3-cd]pyrene	2B	B2
Phenanthrene	3	D
Phenanthrene, 1,4-dimethyl	3	
Perylene	3	
Pyrene	3	D
Pyrene, 1-nitro-,	3	
Pyrene, 1,8-dinitro-,	3	
Quinoline, 8-hydroxy-,	3	

[1] IARC, 1987 classification scheme

1	carcinogenic to humans (*There is sufficient evidence for carcinogenicity in humans*)
2A	probably carcinogenic to humans (*There is limited evidence for carcinogenicity in humans and sufficient evidence for carcinogenicity in experimental animals strengthened by supporting evidence from other relevant data*)
2B	possibly carcinogenic to humans (*There is limited evidence for carcinogenicity in humans in the absence of sufficient evidence for carcinogenicity in experimental animals. It may be used when there is inadequate evidence of carcinogenicity in humans or when human data are nonexistent but there is sufficient evidence in experimental animals. In some instances, an agent for which there is inadequate evidence or no data in humans but limited evidence of carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group*)
3	not classifiable as to carcinogenicity in humans
4	probably not carcinogenic to humans (*there is evidence suggesting lack of carcinogenicity in humans together with evidence suggesting lack of carcinogenicity in experimental animals. In some circumstances, agents for which there is inadequate evidence or no data on carcinogenicity in humans but evidence suggesting lack of carcinogenicity in experimental animals, consistently and strongly supported by a broad range of other relevant data, may be classified in this group*)

[2] USEPA, 1991 classification

B2	Probable human carcinogen
D	Not classifiable as to human carcinogenicity

Table 2.9.2 Weight of evidence carcinogenicity assessments for PAH-containing mixtures (IARC, 1987).

Name	Carcinogenicity Assessment (IARC)
Bitumens	3
Extracts of air or steam refined	2B
Carbon blacks	3
Coal gasification	1
Coal-tar pitches	1
Coal-tars	1
Coke production	1
Creosotes	2A
Iron and steel founding	1
Soots	1
Tobacco smoke	1

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3 DOSE-RESPONSE ASSESSMENT

3.1 Navigation Aid for Dose-Response Assessment

Section 3.1 provides an overview of the organization of the dose-response assessment document. The document consists of four major parts (see figure 3.1.1).

a. **Selection of data** (see section 3.3)

The selection of dose-response assessment approach and the assessment itself are dependent on a wide range of toxicological information and other kind of information. These data are extracted from the world literature. The various data selection processes and the data themselves are outlined in part a of the document.

b. **Rationale for some key decisions** (see section 3.4 and figure 3.4.1.1)

Before we decide on an approach to evaluate the human health risk due to PAH in mixtures, we need to validate a number of key assumptions. The subsequent course of action in the dose-response assessment is strongly influenced by the conclusions arrived at this stage. The following are some of the key questions addressed in part b.

- What is the contribution of the hundreds of PAH typically found in environmental mixtures to the overall toxicity of the mixtures which may contain many other toxic substances? (see section 3.4.3)
- How can the potency of PAH in rodents be accurately extrapolated to humans? (see sections 3.4.4 and 3.4.9)
- Do the PAH potency and the site(s) of the resulting tumors depend on the route of exposure? (see sections 3.4.7 to 3.4.9)

c. **Dose-response assessments** (see sections 3.5 to 3.7 and figures 3.5.1.1 and 3.6.1.1)

In part c of the document, we discuss the two approaches by which we evaluate the contribution of PAH to the carcinogenicity of PAH-containing mixtures (see sections 3.5 and 3.6). The emphasis is on the assessment process itself, since the key rationale for the approaches selected have been established and described in part b. Section 3.7 provides reality checks for the dose-response assessments made. Part c describes the following comparisons.

- Compare our two assessments to each other and to assessments conducted by other authors and agencies.
- Compare our generic estimates with the risk estimates for specific mixtures that were based on human epidemiology data.

Figure 3.1.1 Dose-response assessment process.

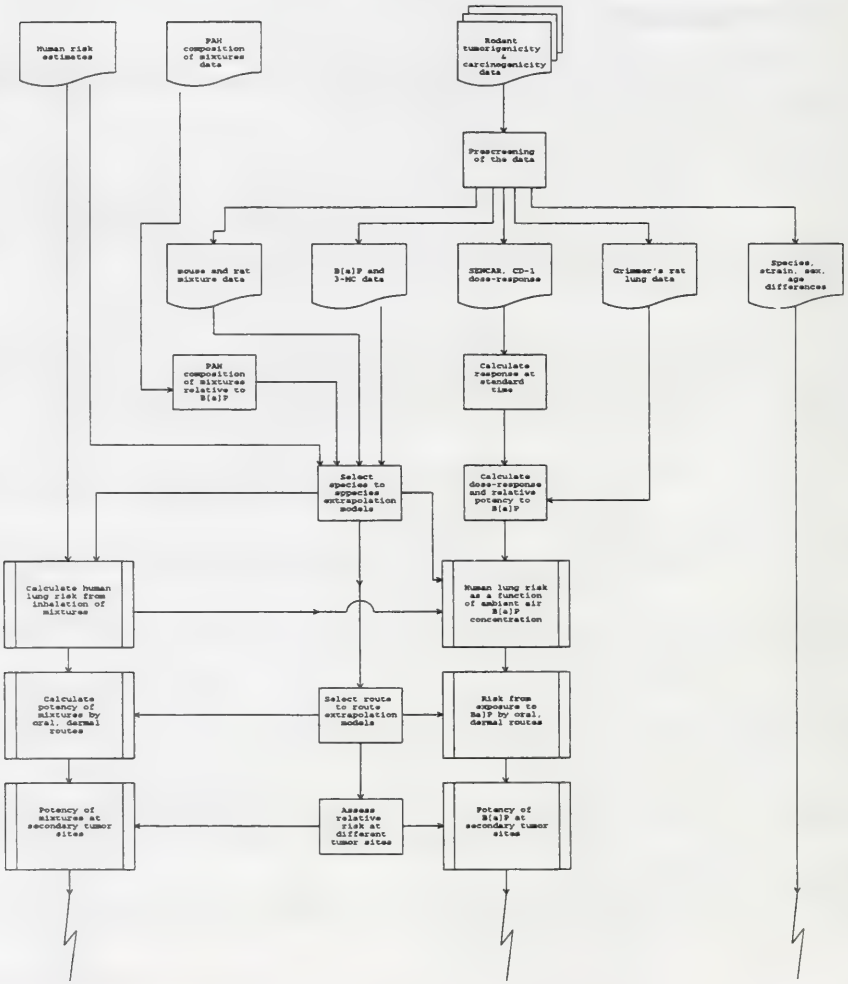
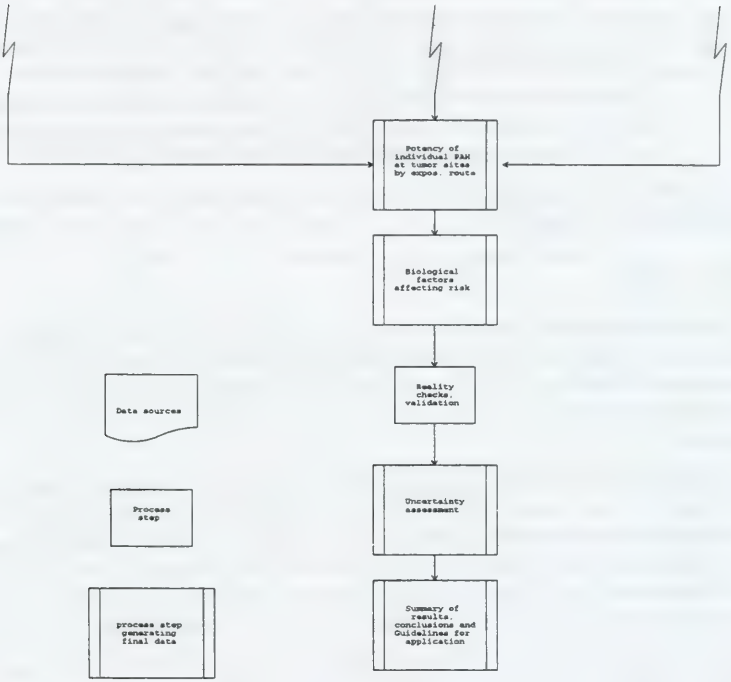

Human risk estimates
PAH composition of mixtures data
Rodent tumorigenicity & carcinogenicity data
Prescreening of the data
mouse and rat mixture data
B[a]P and 3-MC data
SENCAR, CD-1 dose-response
Grimmer's rat lung data
Species, strain, sex, age differences
PAH composition of mixtures relative to B[a]P
Calculate response at standard time
Select species to species extrapolation models
Calculate dose-response and relative potency to B[a]P
Calculate human lung risk from inhalation of mixtures
Human lung risk as a function of ambient air B[a]P concentration
Calculate potency of mixtures by oral, dermal routes
Select route to route extrapolation models
Risk from exposure to Ba]P by oral, dermal routes
Potency of mixtures at secondary tumor sites
Assess relative risk at different tumor sites
Potency of B[a]P at secondary tumor sites

Figure 3.1.1 continued....

Figure 3.1.1 Dose-response assessment process.....continued.


Potency of individual PAH at tumor sites by expos. route
Biological factors affecting risk
Reality checks, validation
Uncertainty assessment
Summary of results, conclusions and Guidelines for application
Data sources
Process step
process step generating final data

- Compare the reported lung and stomach tumor incidences in the general population with the estimated tumor incidences attributable to PAH based on our dose-response assessment.

Part c utilizes data from part a and is based on the rationale developed in part b.

d. **Conclusion** (sections 3.8 to 3.10)

Part d discusses some of the factors which may influence the sensitivity of a subpopulation to PAH, the uncertainty associated with our assessment, and also serves as a guide to the use and interpretation of our dose-response assessment.

The overall relationships between the various components of the dose-response assessment document are shown in figure 3.1.1

3.2 Overview

In conducting the present risk assessment, we frequently encounter situations where key assumptions need to be validated. The data required for validation, however, are often incomplete or unavailable. Under such circumstances, we choose to gather all available information, and to rely on marginal or incomplete data rather than to proceed without any support for our assumptions.

In general, we rely on the weight of evidence concept. When the individual pieces of evidence are by themselves inadequate to validate a given assumption, we take into account the degree to which different pieces of evidence agree with each other. At the end, we make judgments based on the available data, and assign a high degree of uncertainty to a given conclusion or assumption if it is based on inadequate evidence (see section 3.9). However, in many instances, the analysis of available data cannot replace well designed experiments that can verify our conclusions.

The first goal of the dose-response exercise is to assess the risk due to PAH in complex mixtures. This goal is considerably more complex than assessing the risk due to a single specific toxicant. In the overview, the discussion focuses on those issues that differentiate the assessment of PAH in a mixture from the more conventional dose-response assessment for a single identifiable toxicant. Some of the main problems are outlined in section 3.2.1. The two models chosen to address and minimize these problems are outlined in sections 3.2.2 and 3.2.3.

Humans are exposed to PAH primarily from food. Dermal exposure may also be very important in some exposure scenarios, yet these two routes (oral and dermal) have received relatively little attention in existing assessments. Most of the attention has been focused on exposure via the respiratory tract and on lung tumors. Our second goal is therefore to assess the risk from PAH exposure via the inhalation, oral and dermal routes. The issue of how the route of exposure affects the tumor site or the potency of PAH is discussed in sections 3.4.7to 3.4.9, 3.5.5, 3.5.7 and 3.6.4.

As stated above, the main challenge lies in the assessment of PAH toxicity in whole mixtures. There are several dose-response models by which the problem can be addressed, each of which is built on a series of assumptions. Once a particular model is chosen, the risk assessor is largely committed to this set of assumptions. It is therefore important to consider alternatives before one commits to a particular dose-response approach. Our risk assessment is unique in that we put a considerable effort into the evaluation of the assumptions implicit in each model before we select our risk assessment approach. This issue is discussed below and in more detail in section 3.4.3.

3.2.1 Dose-Response Model Selection

Human health risk due to PAH is very difficult to assess because PAH are not found as individual compounds but as mixtures of hundreds of PAH, along with other toxicants. Our goal is to evaluate the contribution of the PAH fraction to the overall risk of the mixture.

It is impractical to quantify the levels of each PAH present in the mixtures. Furthermore, humans are exposed to mixtures of PAH and there are no toxicological data for individual PAH in humans. Therefore, in order to evaluate the risk due to individual PAH, one has to rely on animal data. However, animal data are available for only a few PAH. Since the risk can be assessed only for a handful of PAH, risk estimates based on these PAH will tend to underestimate the overall risk attributable to the total PAH fraction. In addition, the need to extrapolate from animal data to humans brings considerable uncertainty. Possible interactions between the components of the mixture further confounds the assessments based on individual speciated PAH.

An alternative is to evaluate the contribution of PAH to the overall risk of mixtures by treating PAH as a group, rather than as the sum of speciated compounds. The risk of some PAH-rich[10] mixtures has been assessed using human epidemiological data. The availability of human data avoids the necessity to rely solely on animal data for the estimation of human risk. Furthermore, this approach takes into account all PAH as a group. Assessing the risk of PAH as a group, however, has its disadvantages as well. PAH-containing mixtures are generated by many sources characterized by the use of different fuels and combustion conditions. There is no *a priori* reason to expect that the composition and toxicity of these mixtures will be identical to other tested mixtures. Furthermore, the mixtures may contain toxicants other than PAH, making it difficult to identify how much of the mixture toxicity ought to be attributed to the PAH fraction.

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10 We use the term PAH-rich mixtures to describe mixtures that contain high levels of PAH and for which PAH are expected to account for a large proportion of the total carcinogenicity. Coal tar and coke oven emissions, for example, have large PAH fractions (Williams *et al.* 1986), and contain relatively high levels of B[a]P - about 1 µg/mg of extractable organics (Albert *et al.* 1983; Williams *et al.* 1986). In contrast, although cigarette smoke condensate (CSC) contains B[a]P and other PAH, the levels are more than two orders of magnitude lower (Albert *et al.* 1983; Williams *et al.* 1986). CSC therefore belongs to the broader category of PAH-containing mixtures, but cannot be classified as a PAH-rich mixture. The relative composition of CSC is consistent with our findings that CSC does not behave like other PAH-rich mixtures and its B[a]P content accounts for only a small fraction of the mixture's total activity (see section 3.4.6.4).

Over the years, a number of strategies have been developed to estimate the risk attributable to PAH in different mixtures. In general, the risk is estimated by the following methods.

- by combining the risks of speciated PAH (e.g. Toxic Equivalency Factor [TEF])
- from mixtures defined by their source and organic content
- from mixtures defined by their B[a]P content

Most studies utilize one of these three approaches. However, the relative merits of the three methods and the magnitude of risk estimated using the different approaches have not been critically reviewed, and many of the assumptions behind them have not been validated. Thus, even though a number of assessments are available, it is not clear to what extent they agree with each other and how reliable the assessments are.

Our goal is therefore not only to assess risk, but also to compare the three different approaches and to validate the assumptions on which the three models are based. The three approaches are discussed in sections 3.2.1.1 to 3.2.1.3.

3.2.1.1 Estimating Risk of Mixtures by Combining Risk of Speciated PAH

Many risk assessors have estimated the risk of the PAH fraction of mixtures by combining the risk of speciated PAH (Thorslund and Farrar, 1990; Krewski *et al.* 1989; Clement Ass. 1988; Collins and Alexeeff, 1993; Willes *et al.* 1992; Rugen *et al.* 1989; Nisbet and Lagoy, 1992). The human cancer potency of B[a]P was extrapolated from rodent data. Extrapolation usually took the form of a surface area or body weight extrapolation (see section 3.4.4). The potency of PAH other than B[a]P was usually expressed in B[a]P equivalents or toxic equivalency factors (TEFs). The relative potency of PAH to B[a]P in humans was assumed to be the same as the relative potencies derived from rodent experiments.

There are several key problems with this strategy.

a. **Risk is underestimated because too few PAH are considered**

This approach tends to underestimate the risk because out of hundreds of PAH typically found in mixtures, only a handful are considered. Since we also use this approach, we have attempted to minimize the problem in the ways described below.

Resolution:

- We estimated the cancer potency for 209 PAH, several times more than the number of PAH ever evaluated by other authors (see sections 3.5.6 and 3.5.7). Not all of the additional PAH are found in the environment, so expanded environmental monitoring is required to determine the occurrence of each individual PAH in the environment.
- We also relied on another model to provide an alternative estimate of the overall risk (see section 3.6). After comparing the results obtained with the two models, we concluded that the approach based on a handful of speciated PAH underestimates the risk by almost two orders of magnitude (see section 3.7.4).

b. **Uncertainty in species to species extrapolation**

Most risk assessors used rodent data to estimate the potency of B[a]P and other PAH in humans. Although the species extrapolation methods used by many investigators have been partially validated for other non-PAH compounds, the methods may not be appropriate for PAH (see section 3.4.4).

We compared the actual tumorigenic responses in rats and hamsters with responses estimated using surface area or body weight extrapolation methods from mouse data, and found little evidence that the two methods were suitable for extrapolating PAH potency from one species to another (see section 3.4.4). Furthermore, when Thorslund and Farrar (1990) estimated the human potency of mixtures using this method, the potency differed by several orders of magnitude from the potency of similar mixtures based on human epidemiological data. We therefore concluded that there was a need to develop other ways to estimate the potency of B[a]P and other PAH in humans.

Resolution:

- We estimated the B[a]P potency in humans from the potency estimates of PAH-rich mixtures and B[a]P from rodent experimental studies, and potency of the same PAH-rich mixtures derived from human epidemiological data. We assumed that the ratio of the potency of a given PAH-rich mixture in humans and rodents was the same as the ratio of the potency of B[a]P in humans and in rodents. This *relative potency model* has been used by a number of investigators in other context, although it has not been used for the derivation of the human potency of individual PAH from known potency of mixtures. We tested extensively the assumptions underlying the relative potency model using rodent data and concluded that the model was valid for PAH and PAH-rich mixtures in rodents. We

therefore applied the model to estimate B[a]P potency in humans. Like other investigators, we used the relative potency model to evaluate the potency of other PAH in humans by assuming that the relative potency of PAH to B[a]P in rodents and in humans was the same. Our estimates differed slightly from those of others, however, since our B[a]P potency was calculated using both human and animal data, while most investigators depended on surface area extrapolation from animal data only (see section 3.7.2).

- We estimated the cancer risk from exposure to a PAH-rich mixture by combining the risks from speciated PAH. The estimate was then compared to the potency estimate of the same mixture based on human epidemiological data (see section 3.7.4). We found that the speciated approach underestimated the risk by almost two orders of magnitude for two different mixtures. It is possible that the potency of the whole mixture is not entirely due to the PAH, but also due to other compounds present in the mixture. If that is the case, the estimates based on human data would have been overestimates. However, given that both mixtures are rich in PAH, we expect that the main reason for the difference is because the speciated approach does not take into account all the PAH in the mixture.

We have used the additivity approach to estimate human cancer risk from exposure to PAH-rich mixtures (see section 3.2.3). However, recognizing the limitations of the approach, we continued to search for another approach that would better utilize human data and better estimate the risk due to the PAH fraction as a whole. The alternative approach is described below in section 3.2.1.3.

3.2.1.2 Estimating Risk of Mixtures from Organic Content

Albert *et al.* (1983) proposed a different approach, known as the comparative potency model, to assess the risk from exposure to PAH-rich mixtures. According to their model, all mixtures from a homologous source, such as wood smoke (all wood smoke regardless of the type of wood and temperature), have a distinct potency in each animal species, including humans, when the potency is expressed in terms of the mass of organic content in the mixture. Applying the relative potency model which assumes a constant ratio between the potency in humans and in rodent assays for all PAH-rich mixtures, it is possible to calculate the potency of a given mixture provided one knows the potency of the mixture in rodent assays and the relative potency (humans: rodents) for a few homologous mixtures. The key assumption is that one can estimate the potency and composition of a mixture if the type of mixture and the quantity of organics it contains are known. According to the model, diesel emissions would be expected to have a predictable toxicity per unit mass of organic content. However, the potencies of diesel emissions

from different engines were found to vary within a wide range (see Nesnow *et al.* 1982), in contradiction to the comparative potency model. Furthermore, the levels of PAH per ng of organics also vary considerably from one diesel sample to another and the potency of diesel emissions increases with the B[a]P content. Lewtas (1988) has also shown that the tumorigenicity and mutagenicity of diesel emissions correlate with the 1-nitropyrene and 3-nitrofluoranthene content, suggesting that the nitrogenated PAH play a major role in the tumorigenicity of the emissions. Finally, a much larger database for PAH-rich mixtures exists in the literature when their B[a]P content rather than their organic content is recorded. Most regulatory agencies, including the Ontario Ministry of Environment and Energy, routinely monitor the B[a]P content, but not the organic content, of environmental mixtures.

Overall, we conclude that it is preferable to express the potency of mixtures in terms of their B[a]P content, rather than organic content.

3.2.1.3 Estimating Risk of Mixtures from B[a]P Content

The third approach assumes that the tumorigenicity or the PAH fraction of a mixture is proportional to the B[a]P content of the mixture. This approach was originally introduced in the early seventies, when B[a]P was used as an indicator for all airborne pollution. It has been shown, however, that B[a]P cannot serve as an indicator for all compounds . Over the years, B[a]P levels have declined, while the levels of some non-PAH compounds have not. Furthermore, there are numerous sources of air contamination that emit little or no PAH. In the more recent years, B[a]P has been used as an indicator for PAH contamination only. The approach seems to be favoured in Europe, but has not been used in North America. This approach of assessing risk has several attractive features.

- It provides an assessment of the risk due to the PAH fraction of the mixture as a whole.
- It fully utilizes human data and is compatible with risk assessments based on human data.
- It is simple to implement (requires monitoring of B[a]P levels only) and can be, at least in theory, applied to a wide variety of mixtures.

The approach is based on several assumptions which have not been fully validated in previous assessments. The following are the two main assumptions.

- The potency of the PAH fraction of all PAH-rich mixtures when expressed in terms of their B[a]P content is constant.
- The profile of the PAH levels in mixtures relative to B[a]P level does not vary significantly enough to affect the outcome of the risk assessment.

The present report provides evidence that both assumptions are compatible with the available data. Nevertheless, one problem remains: there are no human data suitable to assess cancer risk due to the *PAH fraction* only. Evidence from rodent data indicates that the PAH fraction accounts for the large majority of the risk for a number of PAH-rich mixtures. We therefore make use of the human risk assessments based on exposure to PAH-rich mixtures and assume that the risk attributable to the PAH fraction is the same as the risk due to the whole mixture. Since not all the risk is necessarily due to PAH, the outcome of risk evaluation may overestimate the risk due to the PAH fraction. However, we expect that the overestimation will be relatively small.

We have made use of this approach to develop the *Whole Mixture Model (WMM)* described in section 3.2.2.

3.2.1.4 Conclusions

Sections 3.2.1.1 to 3.2.1.3 outline some of the key considerations prior to the selection of suitable models for the assessment of human health risk due to the PAH fraction of environmental mixtures. Based on the evaluation summarized above, we have selected two models for conducting the dose response assessment for PAH mixtures. Each model has its own advantages and is expected to provide a somewhat different estimate (because of the differing underlying assumptions). In section 3.10.2, we provide recommendations for how to choose between the two models. The models are described in sections 3.2.2 and 3.2.3.

3.2.2 Whole Mixture Model (WMM)

The first model estimates the risk of all unsubstituted *classical* PAH in a mixture, without speciation into individual compounds. We name this model the *Whole Mixture Model (WMM)*. The WMM assumes that the potency of the PAH fraction of mixtures and the levels of individual PAH relative to B[a]P are similar across different mixtures. We have partially validated these assumptions by demonstrating the following.

- The PAH profile of a wide range of mixtures is stable relative to their B[a]P content (see section 3.4.3.2.2.1 and appendix C).
- The potencies of mixtures where PAH are expected to be the major contributors to the carcinogenicity of the whole mixtures are very similar. This is true for both human and rodent data (see section 3.4.3.2.2.2).

Since we assumed that all PAH fractions of complex mixtures had the same potency, we established a *standard* potency for the PAH fraction. We have assigned to the PAH fraction in the complex mixtures human lung cancer risk equal to the risk due to inhalation exposure to coke oven

emissions. The risk from coke oven emissions was reported by USEPA in 1984 and was originally expressed as lifetime risk/(μg of organics/m^3). We converted the reported risk to risk/(ng of B[a]P/m^3) for the present assessment. The rationale for selecting USEPA risk assessment is given in section 3.5.3. Note however, that other risk assessment studies based on other sets of data would have led to quantitatively similar results (see section 3.3.1).

The next step was to estimate the risk at target organ sites other than the lungs and the risk from exposure by other routes. To our knowledge, cancer risk from dermal exposure has not been assessed in humans. Most authors estimated human risk form oral exposure by extrapolation from rodent oral exposure data (see section 3.7.2). In contrast, we assumed that the relative potencies of PAH at different target sites and administered by different routes were the same in humans and in rodents (see section 3.4.6.2).

Next we estimated the relative potency of PAH at different tumor sites using different routes of administration in rodents. We used the rodent relative potency factors and the human inhalation risk to estimate the human risk at different sites due to oral and dermal exposure to PAH mixtures (see section 3.6.4).

3.2.3 Individual PAH Model (IPM)

The second model estimates the risk of the mixture of speciated PAH by summing the risk attributable to individual compound. We call this approach the *Individual PAH Model (IPM)*.

The first step in applying this model is to estimate human risk for B[a]P by the different exposure routes at different tumor sites. Most authors extrapolated B[a]P risk to humans from the rodent inhalation and oral exposure data. The extrapolation was usually done using body weight and surface area extrapolations, or similar methods (see section 3.4.4). It has been generally assumed that the PAH potency in different species can be predicted from their relative body weight or surface area (body mass/surface area to the power of k, where k is a constant which can assume values between 2/3 and 1). These approaches are called body weight and surface area extrapolations and have been validated by different authors for different compounds, but not to our knowledge, for PAH. We therefore attempted to validate the surface area and body weight extrapolations (see section 3.4.4).

Our analysis indicated that surface area or body weight extrapolations did not appear to be predictive of the potency of PAH in one rodent species based on their potency in another species. We concluded that if this method was unconvincing for extrapolation from one rodent species to another, it would unlikely be a reliable tool for rodent-to-human extrapolation. Therefore, we abandoned this method and proceeded with the relative potency approach.

Sections 3.4.6.2 and 3.4.6.3 provides evidence that the relative potency of PAH or PAH-rich mixtures is independent of species and the route of administration. This assumption also implies that the relative potency of individual PAH and PAH-rich mixtures in humans and experimental animals is the same (see equation 3.2.3.1).

$$C = \frac{human\ \ risk\ \ (PAH\ \ 1)}{exper.\ \ potency\ \ (PAH\ \ 1)} = \frac{human\ \ risk\ \ (PAH\ \ 2)}{exper.\ \ potency\ \ (PAH\ \ 2)} = \frac{human\ \ risk\ \ (mixt.1)}{exper.\ \ potency\ \ (mixt.1)} \qquad \text{Equation 3.2.3.1}$$

Using the established potencies of coke oven emissions in both humans and in a rodent assay, and the potency of B[a]P established in the same rodent assay, we calculated the human lung cancer risk due to inhalation of B[a]P by equation 3.2.3.1.

The process of estimating the human risk for B[a]P by the oral and dermal routes is similar to the procedure described in section 3.2.2. We assumed that the potencies of PAH by ingestion and dermal exposure relative to their inhalation potencies were the same in humans as in rodents. Based on this assumption, we estimated the route-specific potencies of B[a]P in humans, using the relative potencies of B[a]P associated with different exposure routes established in the rodent assay.

The final step was to calculate the potencies of other PAH in humans. First, we estimated the potency of individual PAH relative to B[a]P for 209 PAH using rodent data (see sections 3.5.6 and 3.5.7 for details). Human potencies for these PAH were then calculated from the human potency of B[a]P associated with a given route of exposure at a given site, and the relative potency of the PAH to B[a]P. All in all, the human risk due to exposure to each of the 209 PAH has been estimated for the inhalation, oral, and dermal routes of exposure and for each target tumor site.

3.3 Major Data Sets

Our analysis is based on three major data sets: human epidemiological data, experimental rodent tumorigenicity/carcinogenicity data, and analytical data on PAH profile in complex mixtures. The three groups of data are discussed in the subsections below.

3.3.1 Human Epidemiological Studies

We have used human data to estimate the human lung cancer risk attributable to the PAH fraction of environmental mixtures due to inhalation. Several data sets from the world literature are available for assessment (see below). Some of the data sets have been used by more than one risk assessment team in estimating the human risk of lung cancer (see table 3.3.1).

The epidemiological studies mainly involve the occupational exposure of workers to airborne PAH by inhalation. The levels of PAH the study subjects were exposed to are generally much higher than what most people would likely encounter in the environment. The following epidemiological studies are the basis for many of the existing human risk assessments.

- **Gasworker Study**

  This eight year prospective mortality study was conducted by Doll *et al.* (1965). The study involved about 11,500 male workers and retirees in the United Kingdom. The subjects were between the age of 40 to 65 at the onset of the study and had worked for a minimum of five years at a gas plant. About 3,000 cohort members were followed up to four additional years (Doll *et al.* 1972). The subjects were categorized into four groups according to their estimated exposure level. The group with the greatest exposure had a significantly elevated mortality rate from lung cancer. The smoking habits of only about 10% of the workers had been determined. However, the exposed and unexposed workers did not appear to differ much in their smoking habits.

  The exposure data were derived from the study of Lawther *et al.* (1965). The Lawther study recorded the levels of a few PAH (including B[a]P) in several retort houses of gas-works in the UK and provided an estimate of worker exposure in an eight hour work shift.

- **Coke Worker Study**

  The effect of occupational exposure to coke oven emissions was investigated in a series of prospective studies (Lloyd *et al.* 1970; Lloyd, 1971, 1980; Redmond *et al.* 1972, 1976, 1979, 1983; see Bjørseth and Becher, 1986 for review). The studies included thousands of workers from US steel plants that were equipped with coke plants. Some subjects were employed as early as in the early fifties, but the follow-up went well into the mid seventies in some studies.

  The studies showed that the mortality due to respiratory cancer increased in proportion to the coke oven emission levels and the duration of exposure. An increased risk of genitourinary tract cancer (including the kidney) was also reported.

  The smoking history of the study subjects was not known. In addition, because the internal controls of the study (subjects working in steel mills, but NOT in coking plants) were found to experience a higher lung cancer risk than the general population, Lloyd (1980) concluded that the risk derived using this data set could be an under-estimate of the real risk.

The 1984 USEPA risk assessment was based on the coke oven worker study. The level of exposure in the USEPA assessment was expressed in terms of the benzene-soluble fraction of coke oven particulates based on the data of Fannick *et al.* (1972). An alternative method of expressing exposure is in terms of the B[a]P content. The following are some examples.

- WHO (1986) expressed the USEPA lung cancer potency estimate (from Lindstedt and Sollenberg, 1982) in the form of mortality per (ng B[a]P/m^3). They made the conversion based on the finding that B[a]P contributed to 0.71% of the benzene-soluble extract in coke oven emissions.
- WHO (1986) also reported the work of Pott (1985), who calculated the lung cancer risk for a 50 year exposure to 1 ng B[a]P/m^3. Pott had used the data collected by Blome in 1981 from an old coke oven plant in Germany which was believed to have levels that *represented concentrations analogous to* the US installations studied by Lloyd and coworkers.
- The present report expresses the USEPA (1984) risk estimate in terms of the B[a]P content by assuming that the benzene-soluble extract from the coke oven emissions contains the same amount of B[a]P as reported by Albert *et al.* (1983) for the US installations.

The 1984 USEPA risk estimate was the geometric mean of the four *upper confidence limit* (UCL)[11] estimates generated using different assumptions for the lag time between exposure and respiratory cancer death. We derive the *maximum likelihood estimate* (MLE) by taking the geometric mean of the four maximum likelihood estimates calculated from the USEPA data.

USEPA uses the UCL because it is a more conservative approach, erring on the side of safety. We provide some results of UCLs for comparison, but our assessment is based on the MLEs. Our approach is consistent with our intent to present a balanced estimate of risk.

11 This limit represents the 95% upper confidence limit on the slope that best fits the available data. In contrast, the maximum likelihood estimate represents the slope derived as a best fit to the data.

Table 3.3.1.1 Human cancer risk due to inhalation of PAH-rich mixtures.

Lifetime Risk/(ng/m^3 B[a]P)	Derivation Method	Reference
6.6 E-5	Women exposed to *smoky coal* emissions. The slope of the dose response curve was determined by linear regression. We have not been able to obtain a copy of this report (see Slooff *et al.* 1989).	Tuomisto & Jantunen, 1987
1.5 E-3	Women exposed to *smoky coal* emissions. The slope was determined as an average age-specific risk assuming continuous lifetime exposure.	Lewtas *et al.* 1996
4.0 E-6	Gas workers. Dose response by linear interpolation to zero dose and risk. No adjustment for less than lifetime exposure or less than lifetime observation. As a result, this is probably an underestimate by 12 to 14 fold (see Nisbet *et al.* 1983).	Pike *et al.* 1975
4.2 E-4	Gas workers. Dose response by linear interpolation to zero dose and risk. Based on the standardized annual death rate.	Pike, 1983
8.7 E-5	Coke oven workers. Linearized multistage model, based on UCL. Authors converted USEPA (1984) assessment by expressing the risk in terms of B[a]P content, instead of mass of benzene soluble fraction. 0.71% B[a]P content in benzene soluble extract was assumed.	WHO, 1986
5.0 E-5*	Coke oven workers. Linear nonthreshold model.	Pott, 1985
2.3 E-5	Coke oven workers. Linearized multistage model, **MLE** (3.9 E-5 / (µg benzene soluble fraction/m^3)). First, we estimated MLE (expressed in terms of benzene soluble fraction) using USEPA (1984) data and an approach analogous to the EPA's method for estimating UCL. Next, the MLE risk was expressed in terms of B[a]P content by assuming 1.7 ng B[a]P per µg of benzene soluble fraction (from Albert *et al.* 1983).	present report

Table 3.3.1.1 continued....

Table 3.3.1.1 Human cancer risk due to inhalation of PAH-rich mixtures...continued

Lifetime Risk/(ng/m^3 B[a]P)	Derivation Method	Reference
3.6 E-4	Coke oven workers. Linearized multistage model, **UCL** (6.2 E-4 /(μg benzene soluble fraction/m^3)). We changed the USEPA (1984) assessment from an expressing of risk in terms of the mass of the benzene soluble fraction to B[a]P content. 1.7 ng B[a]P/ μg benzene soluble fraction was assumed (from Albert *et al.* 1983).	present report
1.0 E-4	Based on interpretation of existing assessments (Pike, 1983; WHO, 1986 and Tuomisto & Jantunen, 1987)	Slooff *et al.* 1989
2.0 E-6	Roofers and waterproofers. Risk estimate was judged to be low because the control group was expected to have an elevated lung cancer risk as well (Nisbet *et al.* 1983)	Wilson *et al.* 1980
3.2 E-5	Roofers and waterproofers. Based on the linearized multistage model, MLE. Assessment conducted by Thorslund and reported by Albert *et al.* (1983).	Albert *et al.* 1983

* - 50 year exposure, rather than lifetime exposure assumed

- **Study on Roofers and Waterproofers**

  Hammond *et al.* (1976) conducted a prospective mortality study (from 1960 to 1971) on approximately six thousand hot pitch roofers and waterproofers. Increased risk of lung cancer mortality was reported for workers with forty or more years of membership in the *United Slate, Tile and Composition Roofers, Damp and Waterproof Workers Association.*

- **Study on Women Exposed to Smoky Coal Emissions**

  Mumford *et al.* (1987) reported on the prospective lung cancer mortality study conducted on over ninety thousand residents of the Xuan Wei county in the Yunan province of China in the seventies. The county was largely rural. While over 40% of males smoked, women were generally non-smokers (less than 0.1% smoke). Residents usually used either wood, *smoky coal,* or *smokeless coal* for cooking and heating. The fuel was burned in shallow unvented pits and the levels of indoor air pollution were

very high. Men usually spent most of the daylight hours outside of their homes. In contrast, women started the fire and cooked, and therefore were heavily exposed to the smoky coal smoke.

The levels of DNA adducts in the cells obtained by bronchoalveolar lavage (BAL) from individuals exposed to coal smoke were found to be four fold higher than those found in the cells from unexposed persons (Gallagher *et al.* 1993).

The indoor air levels for some PAH were reported by Mumford *et al.* in 1990 and by Lewtas *et al.* in 1996. Since the lifestyle and fuel have not changed over the years, the measurements of PAH levels obtained in the nineties are probably good indicators of the levels in the indoor air in the seventies (the time period of the study).

The epidemiological data and the exposure data described above were used in several assessments and are summarized in table 3.3.1.1. Disregarding the two studies that were judged to be an underestimate by Nisbet *et al.* (1983), most risk estimates lie between 3.2 E-5 and 4.2 E-4, spanning over one order of magnitude.

3.3.2 Rodent Tumorigenicity/Carcinogenicity Data

In our assessment, we have given human data greater weight than animal data because the extrapolation from animals to humans is a difficult process that is associated with considerable uncertainty. Unfortunately, the use of animal data is unavoidable since available human data are too limited to address all the pertinent questions.

Most experimental tumorigenicity/carcinogenicity experiments are conducted by applying PAH to mouse skin and observing the development of skin papillomas and/or carcinomas. Humans, however, are more likely to be exposed to PAH by the oral or inhalation route. Consequently, extrapolation from mouse skin data to other routes of exposure in humans is often necessary. In addition to mouse skin data, our assessment also makes use of the rat lung data generated by Grimmer and his colleagues (1984, 1987, 1988).

Section 3.3.2 provides a general overview of the experimental protocols and of the differences in response due to a difference in the methodology (for further details, see Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons, edited by G. Grimmer, 1983). The overview is not meant to be a detailed description of the laboratory methods used in the studies or to discuss extensively the advantages and disadvantages of different experimental approaches.

3.3.2.1 Description of Protocols

3.3.2.1.1 Topical Application

Most of the tumorigenicity and carcinogenicity data for PAH were generated by topical application. Therefore, we rely on these data for the following.

- estimation of the relative potency of individual PAH and mixtures
- assessment of relative species and strain sensitivity
- extrapolation from rodent data to humans

Tumor Initiation versus Complete Carcinogenicity Assays

There are generally two types of topical application assays, the tumor initiation assay and the complete carcinogenicity assay. The following paragraphs present a brief summary of the mechanism of skin carcinogenesis to facilitate the understanding of the different skin tumor models (for reviews, see Burns, 1989; Boutwell, 1989; Fischer *et al.* 1988).

Skin carcinogenesis process involves three major distinct phases. The first step is initiation. Initiators react with DNA material in the living cells, resulting in permanent, heritable and unexpressed change in the cell. Initiated cells do not usually develop into tumor cells in the absence of promotion. PAH are thought to be primarily initiators, as they induce heritable cell change through interaction with DNA. Initiation by itself has not been demonstrated to be associated with changes in enzyme levels and activity, nor with the appearance of tumors.

Promotion is the second step in mouse skin carcinogenesis. Promotion does not involve changes in DNA, but rather a change in gene expression and a widespread change in enzyme activity. Cellular proliferation is usually part of the promoting process during which the number of altered cells increases. At the tissue level, promotion leads to the appearance of papillomas (benign skin tumors). Some of the papillomas spontaneously progress to carcinomas (malignant tumors). Some require continued promotion to remain in existence, while others disappear once the promotion is discontinued. Most PAH have some promoting capability although their promotion property is not a primary cause for concern.

Progression is the third major step which leads to the development of carcinomas and involves additional mutagenic events.

Compounds that are primarily initiators can be studied by assays that measure either the activity after initiation, after initiation and promotion, or after all three steps have taken place. In practice, most assays that are widely used measure either the initiating effect

selectively or the combined effect of all three steps. The **tumor initiation assay** measures the potency of a compound as a tumor initiator and the **complete carcinogenicity** assay measures the overall potency of a compound as a carcinogen.

The tumor initiation assay involves treating the experimental animals with initiators such as PAH once or a few times several days apart. This treatment is referred to as *initiation*. The subsequent step (*promotion*) involves regular weekly treatment with a promotor such as TPA (a phorbol ester). Typically, omission of either treatment with the initiator or promotor results in a very low tumor incidence. In the tumor initiation assay, papilloma incidence (expressed as % of rodents with tumors) or papilloma multiplicity (number of tumors per rodent) is monitored as the endpoint.

An alternative to the tumor initiation assay is the complete carcinogenesis assay which administers a single dose or repeated regular doses of a single test substance to the experimental animals. Treatment with a second known promotor is omitted. In effect, the test substance serves as both the initiator and the promotor. Carcinoma incidence or multiplicity is monitored as the endpoint. Although assays involving topical application rarely administer a single dose to the animals without subsequent promotion (Bernfeld and Homburger, 1983), single administration of PAH is quite often used in inhalation and ingestion studies.

The tumor initiation protocol is extremely popular, presumably because of the relatively rapid onset of tumors (mostly papillomas, although carcinomas also develop). The results obtained with the tumor initiation assay are reported to be generally comparable to the results from the complete carcinogenesis assay (Slaga and Fischer, 1983). However, this is not always the case. For example, C57BL/6 mice do not develop tumors in the tumor initiation assay (with TPA as a promotor), but do develop tumors in the complete carcinogenicity assay. The difference is probably due to the low efficacy of TPA as a promotor for this strain of mice (see section 3.3.2.2.3). The C57BL/6 mice responses may be more important in understanding the principles of TPA action rather than in conducting PAH risk assessment. A discrepancy of potentially greater consequence, however, is the observation that Nissan diesel emissions (containing PAH) were highly potent in the tumor initiation assay, but ineffective in the complete carcinogenesis assay. We believe that the apparent discrepancy is due to the dose range studied in the complete carcinogenesis assay.

In the tumor initiation assay, the tumor incidence with Nissan emissions was about 50% at 2 mg/mouse (Nesnow *et al.* 1982). To achieve about the same level of incidence with coke oven emissions, a dose 20 fold lower was required (0.1 mg/mouse). In the complete carcinogenesis assay, coke oven emissions induced about 30% incidence with 0.5 mg/mouse/week. This is the lowest dose at which the induced tumor rate was clearly distinct from the control rate. Based on the tumor initiation data that Nissan diesel emissions were

20 times less potent than the coke oven emissions, we predict that Nissan diesel emissions would be able to induce 30 % tumor incidence at a dose of 10 mg/mouse/week in the complete carcinogenesis assay. Since similar or higher dose levels had not been tested, the apparent discrepancy between the two tests is likely due to an experimental artifact.

Tumor initiation assays measure only the initiating potency of tested compounds, and not their promoting efficacy. Since initiation generally takes place at much lower levels of exposure than promotion[12], and since environmental levels of PAH are relatively low, it can be argued that from the perspective of protecting the environment, the tumor initiation effects are more relevant for PAH. Alternatively, it can also be argued that the potency in complete carcinogenicity is more relevant because it takes into account both the initiating and promoting properties of PAH. It should be noted, however, that PAH are generally tested at relatively high doses. The test results tend to favour the promoting properties of the compounds and give a skewed estimate of the overall carcinogenicity of the compounds at environmental levels.

The complete carcinogenesis assay is less popular than the tumor initiation assay because it requires a longer duration of treatment (about 60 weeks to lifetime). Tumor initiation studies usually run for about 30 weeks and rarely for longer then 40 weeks (for more details, see section 3.3.2.2.3). On the other hand, both papillomas and carcinomas are observed in the complete carcinogenicity assay. As a rule, there is a greater proportion of carcinomas in the complete carcinogenicity assay than in the tumor initiation assay.

Our analysis makes use of the data from both types of assays, although we prefer the tumor initiation assay. The tumor initiation assay is more sensitive and more relevant to the low dose effects that may be observable at environmental exposure levels.

Tumorigenicity versus Carcinogenicity

A related issue involves the choice of papilloma (precancerous tumor) versus carcinoma as the endpoint. The present report makes use of both the tumorigenicity and carcinogenicity data. In the tumor initiation assay, papilloma is the primary endpoint. Since PAH at low, environmentally relevant concentrations most likely act as initiators and since initiation is the first step in carcinogenesis, we consider the occurrence of papillomas an appropriate endpoint. In the complete carcinogenicity assays, carcinomas are the common type of tumor and papillomas are less frequent. Both papillomas and carcinomas are therefore

12 For example, about 25 µg of B[a]P per mouse per week is required to induce approximately 50% tumor incidence in complete carcinogenesis assay. However, only between 3 to 13 µg in total is needed to induce the same tumor incidence in the tumor initiation assay. Furthermore, the tumors in the tumor initiation assay develop in half the time they take to develop in the complete carcinogenesis assay (Nesnow *et al.* 1982).

considered in the complete carcinogenicity assays. Although tumorigenicity data and carcinogenicity data could not be compared directly, the relative potencies of PAH to B[a]P based on the two different endpoints are found to be comparable (see section 3.4.6.1).

Tumor Incidence versus Multiplicity

The magnitude of response in the experiments involving topical application is presented in terms of the percentage of mice with tumors (*tumor incidence*) and/or in terms of the average number of tumors per mouse (*tumor multiplicity*).

Experimentally, expressing the magnitude of tumorigenic response in terms of tumor multiplicity has its advantage. It allows the investigator to follow the change in response over a wider dose range. This is because the maximum tumor incidence is 100%, and the dose range at which the incidence is larger than zero but less than 100% is narrower than the dose range at which tumor multiplicity is measurable. On the other hand, it is not possible to identify individual tumor distinctly when a mouse has developed more than six tumors (Don Jerina, private communication). Also, the use of multiplicity as a measure of tumor response assumes that the likelihood of forming an additional tumor is independent of any tumor that is already present. In addition, all the human epidemiological data are presented in terms of incidence, and cannot be directly compared to the multiplicity data.

In order to determine whether the two measures of tumorigenicity yield similar results, we estimated the relative potency of individual PAH to B[a]P using both tumor incidence and multiplicity data. Next, we correlated the potencies obtained by the two methods (see section 3.5.6.2). Since an excellent correlation occurs between the results obtained by the two methods, we use the incidence data throughout the report for simplicity.

3.3.2.1.2 Oral Administration

There are very little data on the effects of orally administered PAH. To our knowledge, no studies have reported the risk due to ingestion of PAH or PAH-containing mixtures in humans and only a few PAH have been administered to experimental animals orally. We used the limited animal ingestion data set in conjunction with other larger data sets, primarily for comparison to establish the relative potency of PAH administered by the oral route. In the experiments summarized in appendix A, PAH were administered in the diet, drinking water or by intubation. The data are too limited to allow comparison of the experimental results using the three methods of oral administration.

3.3.2.1.3 Exposure via Respiratory Tract

Besides topical application, administration of PAH to the respiratory tract is the most common experimental design. We use this data set for the following purposes.

- to establish the relative potency of individual PAH and mixtures containing PAH
- to validate the assumption that the relative potency of PAH is the same, independent of the species and the route of administration
- to estimate the relative sensitivity due to different routes of exposure to PAH

There are three main treatment protocols used in studying the tumorigenicity of PAH when the test substances are administered through the respiratory tract.

1) Inhalation

Inhalation experiments can be conducted in inhalation chambers. Under such conditions, animals receive a significant dose of PAH also through dermal and oral exposure from preening their contaminated fur. An alternative method is to restrict the exposure to the respiratory system by means of tubes attached to the nose of the experimental animal.

Inhalation experiments with PAH are often seen as the most relevant for making inferences about human health risk because the exposure condition is analogous to human inhalation exposure. The model has some disadvantages, however, because the deposition of the inhaled particulate matter within the respiratory tract differ in humans and in rodents as observed in rats and hamsters (Schlesinger, 1985). The differences are not only in the total proportion of particulate that is deposited in the respiratory system, but also in the particle-size-dependent distribution of the deposited particles within the respiratory system. Furthermore, even if dermal exposure is eliminated through the use of nasal-breathing apparatus, some of the particulate matter is inevitably ingested (Saffiotti, 1969) and the ratio of inhaled PAH and ingested PAH in rodents may be different from that in humans.

Thyssen *et al.* (1981), among others, studied the effects of B[a]P due to inhalation. In his experiment, B[a]P was condensed on fine salt particles, which were inhaled (nasal apparatus) by hamsters for 4.5 hours/week for a minimum of 10 weeks. Tumors were detected throughout the upper respiratory tract and the upper digestive tract. No tumors were found in the lungs, presumably because the particle-bound B[a]P did not reach the lungs in adequate quantity. Tumor incidence was dose-dependent.

2) Implantation

Implantation involves injection of a mixture of PAH and a carrier (often equal parts of beeswax and Tricaprylin) into a lobe of a lung. The injection is done under anaesthesia on thoracotomised animals.

3) Intratracheal Instillation

Intratracheal instillation involves placing a catheter or cannula into the trachea of the anesthetized animal and the application of the tested material into the trachea or even into the bronchi. Investigators have used different carriers in administering the tested substance to experimental animals, and found that the carrier itself had a significant effect on the potency of the tested material (see Schneider and Mohr, 1983). Partly because of its simplicity, intratracheal instillation has been used more frequently than the other experimental methods (see Schneider and Mohr, 1983 for more details).

4) Comparison of Three Methods of Administering Test Substances

It appears that implantation of PAH can induce tumors in experimental animals at a lower dose than both inhalation and intratracheal administration. Intratracheal instillation of 15 mg of B[a]P induced a relatively weak response in rats (see table 3.3.2.1.3.1), while about the same magnitude of response was observed at a dose 150 times lower in the lung implantation model. The higher sensitivity of the implantation method may be partly due to tissue response and post-operative traumas (see Schneider and Mohr, 1983), but may also be due to the longer experimental duration in implantation studies.

Table 3.3.2.1.3.1. Effect of intralung implantation and intratracheal instillation on B[a]P potency in rats.

Administration	Dose (mg)	Tumor Type	Incidence	Duration of Treatment	Reference
Intratracheal instillation	15	squamous carc.	22%	17 w	Ishinishi *et al.* 1976
Intrapulmonary implantation	0.1	squamous carc.	31%	22-134 w	Wenzel-Hartung *et al.* 1990
Intrapulmonary implantation	0.1	squamous carc.	29%	99-120 w	Deutsch-Wenzel *et al.* 1983

In hamsters, only 0.7 mg of 3-MC was needed to induce a high incidence of tumor in the lung implantation model (table 3.3.2.1.3.2), while B[a]P required a higher dose (by two orders of magnitude) to elicit the same magnitude of response in the instillation model. The

difference is unlikely due to the difference in potency between the two compounds, since table 3.4.6.2.2.1 shows that B[a]P is about 1 to 5 times more potent than 3-MC. On the other hand, taking into account the use of two different test substances which vary in potencies in the two experiments, the real difference in sensitivity between the implantation and instillation models could have been under-represented by the test results.

Table 3.3.2.1.3.3 summarizes the effect of using the implantation and instillation models in determining the potency of PAH in two animal species, the rat and the hamster. The inhalation model appears to be the least sensitive among the three methods for studying the action of PAH. The implantation model is about two orders of magnitude more sensitive than the instillation model (see table 3.3.2.1.3.2).

Table 3.3.2.1.3.2 Effect of intrabronchial implantation and intratracheal instillation on 3-MC and B[a]P potency in hamsters.

Administration	Dose (mg)	Tumor Type	Incidence	Duration of Treatment	Reference
Intrabroncheal pellet	app. 0.7 3-MC	squamous carc.	61%	> 10 w	Hammond *et al.* 1987
Inhalation	127 B[a]P	upper respiratory tract	34%	≥ 10w	Thyssen *et al.* 1981
Intratracheal instillation	45 B[a]P	mostly squam. carc.	100%	15 w	Saffiotti *et al.* 1968
Intratracheal instillation	15 B[a]P	squamous carc.	15%	17 w	Yamamoto *et al.* 1985
Intratracheal instillation	36 B[a]P	mostly squam. carc.	66%	36 w	Feron *et al.* 1972
Intratracheal instillation	15 B[a]P	mostly squam. carc.	30%	30 w	Saffiotti *et al.* 1972
Intratracheal instillation	30 B[a]P	mostly squam. carc.	66%	30 w	Saffiotti *et al.* 1972
Intratracheal instillation	60 B[a]P	mostly squam. carc.	86%	30 w	Saffiotti *et al.* 1972

All in all, the evidence demonstrating that the intralung implantation model is more sensitive than the intratracheal instillation and inhalation methods to the action of PAH is not very strong. Nevertheless, the evidence justifies caution in making quantitative com-

parison of potencies among PAH based on the data obtained using the three experimental models without taking into consideration the difference generated by the use of different methodologies.

Table 3.3.2.1.3.3 Comparison of PAH potency: implantation versus instillation into the respiratory tract of rodents.

Species	Potency (incidence/mg)			Reference
	Instillation (A)	Implantation (B)	B/A	
rat	1.5	310	210	Ishinishi *et al.* 1976, Wenzel-Hartung *et al.* 1990
hamster	2.2	270*	120	Hammond *et al.* 1987, Saffiotti *et al.* 1972

* - assuming B[a]P is 3 times more potent than 3-MC

3.3.2.2 Prescreening of Data

There is an enormous quantity of publications on the toxicity of PAH in the world literature spanning decades. Despite the apparent richness in experimental data, many key questions relating to risk assessment have not been addressed directly by any investigator. While some studies are suitable for re-analysis to answer questions that the experiment was not originally designed for, it is often needed to combine data from different studies and to make an assessment based on the combined data sets. Unless the data sets match perfectly, however, the differences between two data sets may reflect more methodological differences than differences in the factors which the risk assessor may wish to study. We are therefore careful in the data selection process. This section describes the major blocks of data we extracted from the rodent tumorigenicity literature, and the process used to select and consolidate the data. The combined data sets were then used to address a number of diverse issues, as outlined in sections 3.4 to 3.9.

3.3.2.2.1 PAH-Rich Complex Mixtures

The potency of PAH mixtures in rodents have been studied by a number of investigators (Poel and Kammer, 1957; Hoffmann and Wynder, 1963; Wynder and Hoffmann, 1962; Nesnow *et al.* 1982; Mumford *et al.* 1990). Unfortunately, most investigators have used non-standardized methods, making comparisons between the studies difficult. The following lists a set of mandatory criteria that studies must meet in order to be chosen for analysis. A list of additional desirable features are also provided.

Mandatory Criteria

- Several mixtures were tested using the same protocol.
- B[a]P was tested using the same protocol.
- B[a]P levels in the organic extract of the test mixtures were available.

Desirable Features

- The mass of organic extract in the test mixture was known at each dose level.
- Several individual PAH were tested using the same protocol.

Experiments conducted by two research groups meet the criteria. One group of investigators (Nesnow *et al.* 1992; Mumford *et al.* 1990) tested the PAH-rich complex mixtures using the SENCAR (mouse) tumor initiation assay, while Grimmer and his coworkers tested the mixtures using both the mouse skin and rat lung in their assays (Grimmer *et al.* 1982, 1983, 1984, 1985, 1987, 1988; Grimmer, 1985). The data are summarized in appendix B.

3.3.2.2.2 Tumorigenicity of B[a]P and 3-MC

All risk assessments of PAH involve numerous assumptions. Some of the assumptions involve route- and species-specific sensitivity. Many of the assumptions have not been evaluated, partly because no experiments have ever been designed to validate them.

In our assessment, it is often necessary to pool the data from a number of studies and to make the evaluation based on the pooled data. This pool of data include experiments that were conducted using different protocols in different species via different routes of exposure. The experiments we selected were conducted with B[a]P or 3-MC, the most commonly tested PAH. The studies selected for analysis have most of the following features.

- Studies where 3-MC or B[a]P were administered repeatedly or in a form of sustained release material were given preference.
- 3-MC or B[a]P were administered topically or via oral or respiratory routes.
- Whenever possible, we select publications which reported tumors at specific sites (e.g. skin, lung, forestomach) and of specific cell type (e.g. squamous cell tumors, adenoma).

From each paper selected, we extracted all the basic information including total treatment dose and the observed incidence of tumors at that dose. The description of this second group of studies and of the data is provided in appendix A.

Since most studies administered the test substance at a single dose level, and the dose administered varied from study to study, we expressed the experimental result in a standard format to facilitate comparison between studies. The experimental results were expressed in terms of incidence of tumors per mg of total toxicant administered. In effect, expressing potency as incidence/mg assumes a linear relationship between dose and effect and is an approach consistent with the linear regression method that we used to estimate the dose-response relationship using other data sets (see sections 3.3.2.2.3 and 3.3.2.2.4). Occasionally, the data were sufficient for a full dose response curve. In those cases, we select the pair of dose and incidence data that maximized the incidence per mg (see appendix A).

We used the resulting pool of data to test a number of assumptions concerning route- and species-specific sensitivity (described in some detail in sections 3.4.4 to 3.4.9.). Our data have a number of shortcomings. The limitations and our approach to circumvent the limitations are outlined below.

- **Paucity of data**

  There are few studies available that permit comparison of PAH potency as a function of species and route of administration. Only B[a]P and 3-MC have been tested in multiple species and administered by multiple routes. The data for other PAH are far less complete and are therefore not included in our data set.

  Resolution:

  We have partially validated our findings by comparing the results obtained with B[a]P and 3-MC (see section 3.4.6.2.2 for discussion). We assume that what holds for B[a]P and 3-MC holds also for other PAH.

- **Heterogeneity of experimental design**

  In order to test a number of assumptions, it is necessary to draw comparison from data obtained from different studies and different laboratories. Rarely are we able to find studies where all experimental parameters were the same (except for the parameter under investigation) across studies. Such heterogeneity in experimental design reduces the reliability of the conclusions made.

Resolution:

We select studies that were conducted with methodologies that were as similar as possible, in particular studies that tested the same compound (3-MC or B[a]P) and reported the same type of tumors (e.g. lung squamous cell carcinoma). Results obtained by exposure to a single dose of PAH are not compared to effects due to repeated exposure to the same compound, because the effects of PAH administered in a single or few doses was found to be greater than when the same total dose was administered in many small doses (see section 3.8.2). We also do not pool the results from respiratory tract instillation and implantation studies for comparison because our analysis indicates that rodents are more sensitive to PAH when the animals are exposed by implantation than by instillation (see section 3.3.2.1.3). In many instances, comparison for a specific parameter is conducted using several data pairs. We give greater weight to the conclusion when separate analysis using multiple data pairs confirms the trend.

- **Species difference**

Further problems arise from the different life expectancies of species and the histological/anatomical differences among species. (Example: what should be considered the human equivalent of a forestomach in rodents?)

Resolution:

Whenever possible, we examine if the experimental results obtained in one animal species are consistent with the results observed in another species. Since there are very little data on species other than rodents, our analysis is limited to rodents. If the same observation is found in more than one rodent species, the result would be given greater weight and the same homologous effects would be assumed for humans (see section 3.4.4 for further discussion).

- **Paucity of dose-response curve**

A complete dose-response curve from a single study is rarely available. It is therefore necessary to find a means of comparing dose-response relationships from different studies even if each study administered only one dose level and the dosage was not the same from one study to another.

Resolution:

We express the effects in a standard format, *incidence/mg of total toxicant administered,* and use this measure of potency to make comparison although the experimental conditions might not be the same. The implicit assumption is that the incidence of tumors is linearly dependent on the total dose of toxicant administered to a given animal. This assumption is reasonably consistent with our overall observations.

- **Heterogeneity of endpoints**

  Different species often develop tumors at multiple sites and/or tumors of different types. It is difficult to decide what endpoints to choose for comparison. Should comparison be made between incidence of the most sensitive endpoint or between incidence for the same endpoint? For example, there are limited data on mammary tumors, but good data on forestomach tumors induced in mice by oral PAH administration. In contrast, much of the rat data involve mammary tumors. Using different endpoint in the comparison could in theory affect the conclusion regarding the relative sensitivity of different species.

  Resolution:

  Our analysis indicates the endpoint factor does not, in practice, significantly influence the outcome of potency estimation (see section 3.4.7.4).

Despite the technical difficulties described above, we conclude that comparison between experiments from different laboratories is indeed possible. Partial validation of our approach comes from the observation that the relative potency of PAH in mice, rats and hamsters is similar (see table 3.4.6.2.2.1).

3.3.2.2.3 Mouse Skin Tumor Assays

This data set is intended to serve as a starting point for the estimation of the relative potency of PAH to B[a]P. Consequently, we look for experimental protocols that have been applied consistently to the largest number of PAH. The largest number of individual PAH has been tested in a tumor initiation assay in CD-1 mice (see section 3.3.2.1.1). Although this data set consists of studies conducted by a number of investigators in a number of publications, the assay protocol used by the investigators meets our criteria for consistency (see below).

The same group of investigators also performed a complete carcinogenicity assay (see section 3.3.2.1.1) on C57/BL mice. Again, different investigators used compatible protocols, so we select this smaller data set for comparing the relative potency of PAH in tumor initiation and complete carcinogenicity assays.

Finally, we also look for protocols that have been applied extensively to PAH-rich mixtures as well as individual PAH so as to compare their potencies. We select the tumor initiation assay in SENCAR mice of Nesnow and his collaborators (1982, 1989, 1990).

In the tumor initiation assays, mice were initiated by painting a single dose of a PAH onto their shaved backs. A week later, the mice were promoted with a solution of 12-o-tetradecanoylphorbol-13-acetate (TPA) in acetone, applied twice a week to their dorsal skin. PAH were also administered in acetone in most cases, although in some reports, the solvent contained DMSO[13], THF[14], or traces of ammonium hydroxide in acetone. Upon examination of the data, we conclude that the minor variation in the solvent does not alter the outcome of the experiments and we pool the results of all the studies (see table 3.3.2.2.3.1 for further detail and criteria for data selection). The study results are recorded as tumor (papilloma) incidence (*i.e.* percentage of mice with tumors) and/or papilloma multiplicity (*i.e.* average number of tumors per mouse). Either way, both measures are indicative of the tumor-initiating capacity of PAH and both are provided in the present report.

In complete carcinogenicity experiments, PAH was painted onto the shaved dorsal skin of a mouse, usually once a week over a period of several months. The results are expressed as tumor incidence, i.e. the percentage of mice with tumors (carcinomas).

The skin-painting experiments in different laboratories differed slightly in experimental details. The importance of the difference in rodent strain has been discussed in section 3.4.6.1. The two types of initiation-promotion methods are considered separately because of strain difference and also because the TPA dosage differs in the two methods. Table 3.3.2.2.3.1 provides a summary of the experimental methods used in the dose-response data set.

We have extracted the following information for our database.

- Dosing schedule and total dose of PAH
- Experiment duration (from the time PAH was first administered to the time of observation)
- Magnitude of response given as tumor incidence or tumor multiplicity

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13 dimethyl sulfoxide

14 tetrahydrofuran

Table 3.3.2.2.3.1. Criteria for inclusion of mouse skin-painting protocols for data analysis.

Strain	Sex	Age (weeks)	PAH Volume /application	TPA /application (nmol)	TPA Volume /application	Reference
CD-1	F	7-8	200 µL	16	200 µL	Buening *et al.* 1980
SENCAR	F	7-9	200 µL	3.2	200 µL	Iyer *et al.* 1980
C57BL	F	4-5	50 µL	none	none	Levin *et al.* 1977

- Reference to the report from which the data were extracted

The database consists of 64 publications, including data on 197 compounds, and contains 829 records. One of the major problems encountered was the different nomenclature used by different authors. Since our purpose is to compare/combine data referring to the same compound, we have to identify the various synonyms referring to each PAH in our database. The synonyms were standardized using the Chemical Abstract Service (CAS) numbers. For some compounds, CAS numbers were not available and we designed special codes for them. The synonyms for individual PAH and their CAS numbers, as well as a general discussion of PAH nomenclature are included in appendix E.

Most studies reported results only for a single experimental duration. Although most of the experimental parameters were compatible from study to study, the duration of the experiments often differed. In order to compare results, it becomes necessary to model the magnitude of response at a fixed *standard* time given the magnitude of response at a different time point (for a discussion of the time standardization, see section 3.5.6).

Some of the studies (23 studies) reported the entire time-course of PAH-induced tumor development. We have created another database for these studies and used them to develop a mathematical function describing the relationship between time and the magnitude of response (refer to section 3.5.6 for details). The structure of this database (with 1290 records) is the same as the database described above.

3.3.2.2.4 Rat Lung Tumor Assays

Rat lung data are used for the following purposes.

- to develop estimates of risk for individual PAH
- to compare the relative potencies of PAH to B[a]P in rat lungs and mouse skin
- to compare the potency of mixtures and individual PAH in the rat lung model

The data were extracted from the reports of Grimmer and his coworkers (1984, 1987, 1988) and the testing was done using a consistent methodology.

Female Osborne rats were used in the experiments. The space between the fifth and sixth ribs was surgically opened in nembutal-anesthetized mice and 50 µl of a mixture containing the test compound and equal parts Trioctanoin and beeswax, preheated to 60 °C, was injected into the left lobe of the lung. The animals were autopsied at death.

3.3.3 PAH Composition of Mixtures

Section 3.3.3 establishes the PAH profiles of different environmental mixtures. Relative PAH levels were developed by dividing the levels of individual PAH by the level of B[a]P in each mixture. We name the list of relative concentrations as the PAH profile of a mixture.

In establishing the PAH profile, we included only studies that tested a relatively large number of PAH. Two sets of data were summarized. The first set covers source mixtures, such as diesel emissions, coke oven emissions, and coal tar, and is summarized in section 3.3.3.1.

In addition, we summarized the average profiles of PAH in soils, sediments and air. The details are discused in the exposure assessment document. Section 3.3.3.2 provides only a summary of the different profiles.

3.3.3.1 Source Mixtures

In total, the present report has examined 94 mixtures. We classified mixtures into mixture types (such as diesel emissions, coke oven emissions, coal tar, etc.) and identified individual PAH that were most commonly tested in the mixtures. Table 3.3.3.1.1 lists the fifteen most commonly tested compounds in the mixtures and the number of mixture types for each PAH listed.

Using the data grouped by mixture type, we calculated the level of each PAH relative to B[a]P. In the original publications, the units of measurement differed from study to study. However, such difference does not affect the outcome of the present exercise (see appendix C for details).

Table 3.3.3.1.1 Number of mixture types for which analytical data were reported for a given PAH. Compounds included are the most frequently reported PAH.

Compound	# of Mixture Types*
Anthracene	7
Phenanthrene	7
Fluoranthene	14
Pyrene	14
Benz[a]anthracene	12
Perylene	12
Benzo[e]pyrene	11
Benzo[a]pyrene	12
Benzo[ghi]perylene	12
Dibenz[a,h]anthracene	10
Coronene	10
Indeno[1,2,3-cd]pyrene	9
Anthanthrene	5
Chrysene/Triphenylene	12
Benzofluoranthenes	14

* - A total of 14 mixture types were considered.

When there were more than two values for the relative PAH levels for a given source mixture type, we determined the geometric mean of the relative levels for each PAH. In addition, the upper and lower 95% confidence limits and the confidence range (CR) were calculated for each PAH within a mixture type, provided that at least three sets of data were available for a given mixture type. The confidence ranges are presented in table 3.3.3.1.2.

The confidence range (*CR*) was used as a measure of the range the mean of the relative levels a given PAH will assume about 95 out of a hundred times. The CR was determined by dividing the Upper 95% confidence limit by the Lower 95% confidence limit (see appendix C for details). An assessment of the variability of the profiles of mixtures is summarized in section 3.4.3.2.2.1.1.

Table 3.3.3.1.2 Confidence ranges of relative levels of individual PAH (compared to B[a]P) in different mixture types.

Compound	C.O.	C.T.	CFPP	C.S.	O&F	W.S.	D.E.	G.E.	R.A.	P.A.
Anthracene	3.2	440	830			7.6		1.8		
Phenanthrene	1.3	32	27			2.7		2.3		
Fluoranthene	2.7	4.5	3.2	43	4.9	1.4	6.1	3.5	5.7	5.2
Pyrene	2.6	280	37	18	5.6	1.5	5.3	4.0	19	2.7
Benz[a]anthracene	19		2.8	32	160	2.0	9.0		130	8.5
Perylene	2.3	27		23	8.2	31		4.8	4.6	5.9
Benzo[e]pyrene	1.3	5.5	3.3	35	1.6	1.5	2.2	1.8		
Benzo[ghi]perylene	8.8			12	32	2.7	8.9	1.7	5.1	2.8
Dibenz[a,h]anthracene				310	3.2		2.4			
Coronene	7.8			7.1	730		3.2	1.9		
Indeno[1,2,3-cd]pyrene					7.4	1.9	7.0	1.8		
Anthanthrene	8.7							2.1		
Chrysene/Triphenylene	2.1	12	430	42	25	4.3	7.3	2.4	23	6.1
Benzofluoranthenes	5.7		2.0	28	7.1	5.0	3.4	1.7	7.7	120

C.O. = Coke Ovens
C.T. = Coal Tar
CFPP = Coal-fired Power Plants
C.S. = Coal Stoves
O&F = Open and Fireplace Burning
W.S. = Wood Stoves
D.E. = Diesel Emissions
G.E. = Gasoline Emissions
R.A. = Roofing Asphalt
P.A. = Paving Asphalt

3.3.3.2 All Mixtures

The stability of the PAH profiles across the various types of samples in various environmental media (soil, sediments, air and source mixtures) were examined. Mean PAH levels were determined for each of the media and the values were, in turn, used to calculate the geometric mean, upper and lower 95% confidence limits and the confidence range value for each PAH taking all media into consideration. The statistics enable an evaluation of the stability of the PAH profiles across the four media and are shown in table 3.3.3.2.1.

Examination of the data shows that the mean values for the four smallest PAH (phenanthrene, acenaphthylene, acenaphthene, fluorene) compounds are higher in the air than in other media (see table 3.3.3.2.1). This is because soil, sediment and source mixture studies measure mainly PAH that are bound to particulate matter. On the other hand, studies of PAH levels in the air measure the combined levels of volatilized PAH and particulate-bound PAH. For the small PAH, more molecules are volatilized and less are bound to particulate. In contrast,

the majority of the PAH examined (with larger molecular weight) are mostly associated with the particulate matter in the air, therefore the air samples and samples in other media of these PAH are similar.

Table 3.3.3.2.1 Geometric means of concentrations of individual PAH relative to B[a]P in whole air, soils, sediments and source mixtures.

Compound	Whole Air[a]	Soils	Sediments	Source Mixtures
Anthracene	2.9	3.4	0.47	3.9
Phenanthrene	60	2.3	3.6	18
Fluoranthene	18	3.9	3.2	4.9
Pyrene	12	2.1	2.4	4.5
Benz[a]anthracene	0.97	0.96	1.4	1.8
Perylene	0.26	0.16	1.4	0.51
Benzo[e]pyrene	1.4	0.46	1.4	1.0
Benzo[ghi]perylene	1.6	1.0	0.96	0.98
Dibenz[a,h]anthracene	-	0.20	0.30	0.35
Coronene	-	-	0.45	0.35
Indeno[1,2,3-cd]pyrene	1.4	0.64	1.2	0.51
Anthanthrene	0.17		0.19	0.49
Chrysene/Triphenylene	3.0	2.0	1.2	2.3
Benzofluoranthenes	3.6	1.4	2.4	1.6
Acenaphthylene	3.6	0.39	0.35	-
Acenaphthene	4.6	0.61	0.72	-
Fluorene	13	3.8	1.2	-

[a] - Whole air data from T. Dann, Environment Canada.

A summary of the annual air levels is available for a number of PAH from various locations throughout Ontario (MOEE, G. Diamond). Based on the data, mean levels (relative to B[a]P) were determined for both the particulate bound portion and the total particulate/air samples for each of the PAH listed (see table 3.3.3.2.2). The data clearly show that the levels of volatile

compounds in the air profile are consistently higher than in the other three (non-air) profiles when the whole air sample (particulate and air) is taken into consideration. On the other hand, the PAH profiles for the higher molecular weight PAH in whole air samples are similar to other media. When air monitoring only measures the particulate fraction (see table 3.3.3.2.2), the PAH profiles are comparable across all four media for all PAH examined.

Statistical analysis of the data in support of the above qualitative analysis is provided in section 3.4.3.2.2.1.

Table 3.3.3.2.2 Geometric means of PAH levels relative to B[a]P in air particulate, total air, soils, sediments and source mixtures.

Compound	Air Particulate [a]	Total Air[a]	Soils	Sediments	Source Mixtures
Anthracene	1.1	5.6	3.4	0.47	3.9
Phenanthrene	2.2	49	2.3	3.6	18
Fluoranthene	3.4	13	3.9	3.2	4.9
Pyrene	2.8	8.8	2.1	2.4	4.5
Benz[a]anthracene	1.0	0.99	0.96	1.4	1.8
Perylene	0.36	0.62	0.16	1.4	0.51
Benzo[e]pyrene	2.0	1.8	0.46	1.4	1.0
Benzo[ghi]perylene	1.2	0.91	1.0	0.96	0.98
Dibenz[a,h]anthracene	-	-	0.20	0.30	0.35
Coronene	0.26	0.076	-	0.45	0.35
Indeno[1,2,3-cd]pyrene	1.4	1.0	0.64	1.2	0.51
Anthanthrene	-	-	-	0.19	0.49
Chrysene/Triphenylene	2.9	2.4	2.0	1.2	2.3
Benzofluoranthenes	7.2	4.7	1.4	2.4	1.6
Acenaphthylene	0.35	11	0.39	0.35	-
Acenaphthene	0.81	19	0.61	0.72	-
Fluorene	0.87	23	3.8	1.2	-

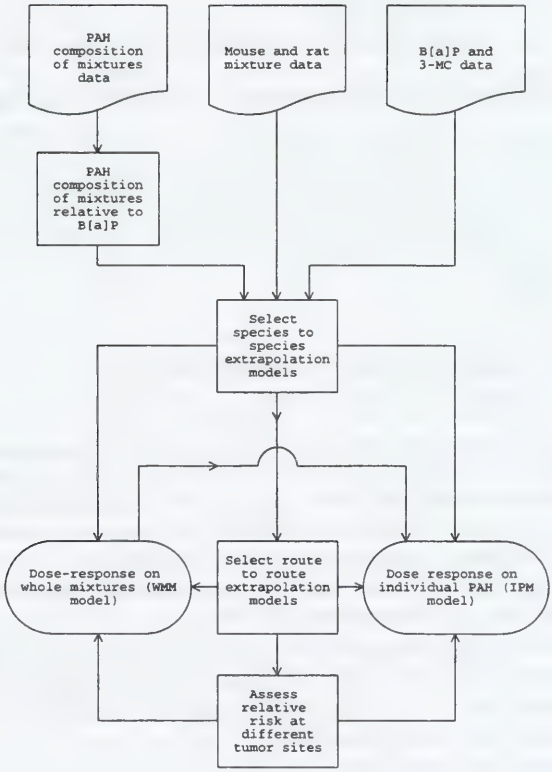
[a] - air data from G. Diamond, Ontario Ministry of Environment.

3.4 Rationale for Some Key Decisions

3.4.1 Navigation Aid

This is the beginning of part b which describes the *rationale for some key decisions.* Figure 3.4.1 assists in navigating through part b and relates part b to other parts of the document.

Figure 3.4.1 Rationale for some key decisions - a process flow diagram


PAH composition of mixtures data
Mouse and rat mixture data
B[a]P and 3-MC data
PAH composition of mixtures relative to B[a]P
Select species to species extrapolation models
Dose-response on whole mixtures (WMM model)
Select route to route extrapolation models
Dose response on individual PAH (IPM model)
Assess relative risk at different tumor sites

3.4.2 Introduction

Before one can perform the dose response assessment, a number of key issues have to be resolved. These issues are described as follows.

- Selection of alternative methods for the conduct of risk assessment (e.g. Should the assessment be based on the sum of the risks of individual components of the mixture or based on the whole mixture?)
- Species to species extrapolation
- Route to route extrapolation
- Selection of tumor sites to be considered in the assessment

3.4.3 Dose-Response Assessment - Available Alternatives

PAH are found in the environment as components of many complex mixtures. Typically, the complex mixtures contain a wide range of PAH and other compounds. Numerous methods for assessing the risk of this large family of compounds in different environmental media (air, food, soil, etc.) have developed over the years. In general, there are two main approaches: summing the risks of individual PAH, and analyzing the risk of the mixture as a whole. Each approach has its own advantages.

3.4.3.1 Summing Risks of Individual PAH

The potency of a mixture can be estimated as a function of both the potency and quantity of its individual components. The best known model based on this principle was developed by Thorslund and Charnley in 1988. The tumorigenicity of each individual PAH is expressed in terms of *B[a]P equivalents*, and is derived from the potency estimate of the PAH relative to the potency of B[a]P. Tumorigenic risk of a particular mixture is estimated from the sum of the B[a]P equivalents in the mixture. The mixture is assumed to have the same potency as if B[a]P equivalents were B[a]P present at the same concentration. Thorslund and Charnley named this approach the "comparative potency approach".

The same approach has also been recommended by other authors, including Rugen *et al.* (1989), Krewski *et al.* (1989), Nisbet and LaGoy (1992), Schoeny *et al.* (1991), as well as Schoeny and Poirier (1993). In the present report, the model of Thorslund and Charnley will be referred to as the *Individual PAH Model (IPM)*.

The IPM model is compatible with existing regulatory approaches. It is relatively simple and versatile, because it can be applied to any mixture and to any medium. The main problem is the uncertainty associated with the resulting potency estimates. There are no quantitative human toxicology data for individual PAH and extrapolations from animal data are associated with considerable uncertainty. The model assumes that the risks associated with individual components of the mixture (including PAH) are additive (see Krewski *et al.* 1989). This assumption may not be always true, however, as mixtures contain compounds that are initiators as well as promotors, and it is unlikely that the effects of compounds acting through different mechanisms are additive.

Perhaps most importantly, the complex mixtures contain hundreds of PAH, yet animal toxicological data are available for only a few compounds. Hence, the toxicity of the great majority of PAH is unknown. It is possible that some PAH are strongly carcinogenic and contribute significantly to the overall toxicity of the mixtures. It is therefore likely that the IPM model significantly underestimates the risk associated with the PAH in the mixture. The likelihood of underestimation is supported by the analysis of Thorslund and Farrar (1990) who estimated and compared the potency of the mixtures as a whole, the potency of the PAH fraction containing PAH with four or more rings, and the potency attributable to the sum of eight commonly studied PAH. The authors reported that for three out of four mixtures considered, the individual PAH together contributed to less than five percent of the toxicity of the PAH fraction.

We use *Individual PAH Model* (IPM) as one of the two models in our dose-response assessment. The details of the assessment are outlined in section 3.5. An alternative method of assessing the risk of a mixture is to estimate the risk of the mixture as a whole, rather than summing the risks of its components. The alternative method is discussed in the next section.

3.4.3.2 Dose-Response Assessment of Mixture as a Whole

An alternative method for assessing the risk due to a mixture estimates the potency of the mixture as a whole and is inherently based on the assumption that the potency of the PAH fraction of complex mixtures can be predicted from the content of an appropriate indicator. The potency of the mixture can theoretically be expressed in terms of the mass of the organic extract in the particulate, the mass of the PAH fraction of the extract or the mass of an indicator compound such as B[a]P. Since the available monitoring data are not sufficient to allow the use of the *PAH fraction* as an indicator, one has to choose between using the mass of *extractable organics* or of a PAH such as B[a]P as the indicator.

USEPA (1984) calculated the risk from exposure to coke oven emissions in terms of benzene-extractable organics. Albert *et al.* (1983) expressed the risk from coal tar fumes and cigarette smoke in a similar fashion. Albert *et al.* (1983) further suggested that the same expression could be used to describe the potency of a wide range of mixtures. Since this method

has also been implemented in a number of other studies (Lewtas, 1985, 1988; and Nesnow, 1990), we will first examine the option of using organic extractables as an indicator for the toxicity of the mixture.

3.4.3.2.1 Organic Extractables as Indicator

The proponents for using organic extract as the indicator of toxicity for a mixture implicitly assume that the composition and the potency of the mixture from a given source are similar and do not change significantly as a result of a difference in fuel or operating conditions. Thus Albert *et al.* (1983) and a number of investigators referred to a single potency for each source type, e.g. coke oven emissions, diesel emissions, roofing tar, etc. This is practical only if mixtures from a similar source can be shown to have comparable potencies.

We tested the model of Albert *et al.* (1983) using the data from Nesnow and his collaborators. Nesnow *et al.* (1982) tested B[a]P and a number of mixtures under identical experimental conditions. His data on a series of emissions generated by different diesel engines are most relevant for testing the model assumptions. If the model is appropriate, and the potency of mixtures can be predicted from knowing the source type and the quantity of organic extract, then all diesel emissions should be approximately equipotent provided that the potency is expressed in terms of extractable organics.

In Nesnow's experiments, each group of mice was first initiated with the organic extract from one of the mixtures by topical application (see section 3.3.2.1.1 for a discussion of tumor initiation assays). All mice were subsequently promoted with repeated applications of a promotor (TPA - a phorbol ester). Although both tumor multiplicity and tumor incidence data were recorded by the author, only tumor incidence was used in the present assessment (see discussion of incidence and multiplicity in section 3.5.6.2).

Initially, we plotted all the data and inspected them visually. For some mixtures, the response did not appear to be dose-dependent and for others, the group exposed to the mixture did not seem to be significantly different from the control group. Consequently, we used some of the statistical tests provided in the ToxRisk program to identify the inactive mixtures.

- **Fisher's Exact Test**
  Fisher's Exact Test tests if the tumor incidence in the *control* group differs significantly from the *treated* groups. This test does not require the effects to be dose-dependent. It is sufficient if one of the *treated* groups is significantly different from the *control*.

- **Mantel-Haenszel Trend Test**
  Mantel-Haenszel Trend Test compares the tumor incidence in all experimental animals groups (including the control). The test is positive when the magnitude of the effect is dose-dependent. Such trend has been observed for some of the less potent mixtures.

For a particular mixture to be considered *active*, the tumor incidence for at least one test group must be significantly different from the control group ($p \leq 0.05$) **or** Fisher's Exact Test must show a significant deviation for at least one treatment group. Mixtures judged inactive by both tests were assigned a potency of zero. For the remaining mixtures, the slope of the dose-response curve was estimated by linear regression, and the potency determined from the slope of the dose response curve (see section 3.5.6.2 for a detailed discussion of the experimental protocol and its rationale).

As stated earlier in the section, the implicit assumption of the model of Albert *et al.* (1983) is that the mixtures from similar sources have a similar potency and perhaps a similar composition. The available data for diesel emissions do not support this assumption (see table 3.4.3.2.1.1). The mixtures range widely in their potency and in B[a]P content (per mg of organics) even though they are all derived from the same type of sources (diesel). For example, the B[a]P content of one mg of diesel emission organics differs by 600 fold from one brand to another. Thus, the mass of extractable organics is a poor predictor of the B[a]P content of a given mixture.

Table 3.4.3.2.1.1 B[a]F content and potency of diesel emissions in tumor initiation assay. Results are calculated from the data of Nesnow *et al.* (1982). For experimental details, see section 3.3.2.1.1.

Source	Incidence /mg of organics	ng B[a]P/mg organics
Nissan	9.7	1200
Oldsmobile	0	2.0
Caterpillar	0	2.0
Mercedes	2.0	Not available
VW Rabbit	2.9	26

Consider specifically the Nissan and Oldsmobile data. Based on the content of organic extractables, the two mixtures should be equipotent based on the model of Albert *et al.* (1983). However, the two mixtures are not equipotent. When the potency is expressed in

terms of organic extractables, Nissan emissions are among the most potent mixtures (see table 3.4.3.2.2.2.1), and Oldsmobile emissions are not active in the tumor initiation assay. An examination of the B[a]P content reveals a possible explanation. The Nissan organic extract contains about 600 times more B[a]P than the organic extract from Oldsmobile diesel emission. The low levels of PAH likely present in the Oldsmobile extract are probably insufficient to induce a response in the tumor initiation assay.

Based on the evidence presented above, we conclude that the mass of organic content is a poor predictor of the potency of mixtures, even if they originate from similar sources. We next examine B[a]P as a possible indicator.

3.4.3.2.2 B[a]P as Indicator

A number of researchers and regulatory agencies have explored the use of B[a]P as a surrogate or indicator compound for PAH in a mixture (see Nisbet *et al.* 1983 for review of reports from 1972 to 1982; WHO, 1987; Slooff *et al.* 1989). In this context, the potency ascribed to B[a]P is not thought to be the potency of the compound itself, but rather an estimate of the potency of the PAH fraction of the mixture. We therefore denote B[a]P as *B[a]PS* to represent the whole PAH fraction for which B[a]P serves as the surrogate.

B[a]PS was initially proposed as an indicator for all urban pollution, and a number of assessments were based *primarily (or entirely) on studies in which the general population was exposed to the ambient air, and B[a]P was used as an index of exposure for a wider variety of compounds* (Nisbet *et al.* 1983). Since the relative proportion of B[a]P and other PAH has been declining with respect to other compounds, such as VOCs, in the ambient urban air over the years, B[a]P does not appear to be a suitable indicator compound for the entire mixture. In fact, B[a]P levels do not vary proportionately with the levels of some substituted PAH in ambient urban air (Albert *et al.* 1983). Because of these reasons, USEPA decided to use organic extractables instead of B[a]P, as the indicator in its risk assessment on coke oven emissions and coal tars (USEPA, 1984, 1984b).

Wilson *et al.* (1980) questioned the usefulness of B[a]P in predicting the levels of other PAH in the same source mixture. They showed that the relative levels of PAH varied at different parts of the emission plume from coal-fired power plants. The observed phenomenon was thought to be either due to the formation of PAH in the plume or the condensation of PAH onto the particulates at different rates. While there is undeniably a difference between the relative proportions of individual PAH in the stack and the plume of the emission, the difference is typically about one to three fold (see table 3.4.3.2.2.1). We consider a difference of such magnitude would not represent a large source of uncertainty in our risk assessment in comparison to other sources of error (see section 3.9).

Table 3.4.3.2.2.1 Relative concentration of PAH in the plume of the emission and the stack of coal-fired power plants. Data are from Wilson *et al.* 1980.

PAH	Levels of PAH - relative to B[a]P content		
	in stack	in plume (0-5 miles)	in plume (5-10 miles)
fluoranthene	0.21	0.31	0.54
pyrene	1.3	3.7	1.1
benzo[a]anthracene	5.3	14.3	1.8
chrysene	3.7	4.2	1.3
perylene	0.15	0.43	1.1
benzo[e]pyrene	0.75	0.98	1.6
benzoperylene	0.92	0.80	-

Other authors have explored the possibility of using the profile of PAH within a mixture as the marker for identifying the source of the mixture (Vogt *et al.* 1986; Gordon and Bryan, 1973; Greenberg *et al.* 1981). While there may be some difference in the PAH profiles of mixtures depending on their sources, the actual difference in the levels of PAH with four or more rings relative to B[a]P is relatively small (see section 3.4.3.2.2.1).

We agree that B[a]P should not be used as an indicator for all urban air pollution, because it is not necessarily a good predictor for other toxic non-PAH compounds which may also be present in the air. On the other hand, we believe that B[a]P is a good indicator for other PAH in complex mixtures. Although there are differences in the PAH profiles of different PAH-rich complex mixtures, for at least a dozen or so of the most commonly assayed unsubstituted PAH, the differences are too small to have a large impact on the outcome of the overall risk assessment. Nevertheless, there are exceptions, such as 1-nitropyrene (see data presented by Albert *et al.* 1983). Data analysis in support of our conclusion is presented in the following subsections.

3.4.3.2.2.1 PAH Profile of Mixtures

The object of the analysis is to determine the extent by which PAH profiles vary from mixture to mixture. Relative PAH levels were developed by dividing the levels of individual PAH by the level of B[a]P in each mixture. The result is referred to as the PAH profile of the mixture. If the levels of the various PAH are proportional to the B[a]P

level in the mixture, it would be sufficient to monitor B[a]P alone as an indicator for other PAH. The data used for the analysis are summarized in sections 3.3.3.1 and 3.3.3.2 and the process is outlined in the next subsection.

3.4.3.2.2.1.1 Methods and Results

We extracted PAH profiles for source mixtures from the published literature and based the profiles for environmental mixtures mainly on Ontario and Canadian monitoring data (see sections 3.3.3.1 and 3.3.3.2). The data were grouped by mixture type and the PAH profile was determined for each mixture.

Subsection A examines the data on source mixtures to determine whether mixtures from different sources have similar profiles. The exposure assessment document performs a similar analysis of PAH profiles in soils, sediments and the ambient air. The analysis is briefly summarized in subsection B. The final step compares the profiles of source mixtures, soils, sediments and the ambient air to determine if the profiles are sufficiently similar to justify the assumption that all mixtures have the same PAH profile. This aspect is discussed in subsection C.

In all of the analyses, we expect some variation in the PAH profile within and between mixture types. The purpose of the analysis is to determine if the differences are large enough to significantly affect the outcome of the risk assessment on the mixtures. We have used *confidence range* (CR) as a measure of the relative variation in PAH levels.

The CR of the PAH level relative to B[a]P content for each PAH was determined by dividing the upper 95% confidence limit by the lower 95% confidence limit of the ratio of PAH to B[a]P levels in the mixture. CR provides a measure of the range the mean of the relative level of a given PAH assumes 95 out of a hundred times.

A) PAH Profiles of Source Mixtures

The confidence ranges were calculated for each PAH within a source mixture type and are shown in table 3.4.3.2.2.1.1.1. For each type of source mixtures, we determined the number of PAH for which the CR exceeded a value of 50. A cutoff value of 50 was selected. A CR value of less than 50 suggests that the relative level of PAH is sufficiently stable for the mixture type so that the B[a]P level in a given source mixture could reasonably predict the level of the other PAH in the mixture. It also means that the variation in PAH levels is unlikely to significantly alter the risk estimate of the mixture as a whole (for further details, see appendix C).

Table 3.4.3.2.2.1.1.1 Confidence ranges of PAH in source mixtures and number of PAH for which CR exceeds 50 in each mixture type.

Compound	C.O.	C.T.	CFPP	C.S.	O&F	W.S.	D.E.	G.E.	R.A.	P.A.
Anthracene	3.2	440	830			7.6		1.8		
Phenanthrene	1.3	32	27			2.7		2.3		
Fluoranthene	2.7	4.5	3.2	43	4.9	1.4	6.1	3.5	5.7	5.2
Pyrene	2.6	280	37	18	5.6	1.5	5.3	4.0	19	2.7
Benz[a]anthracene	19		2.8	32	160	2.0	9.0		130	8.5
Perylene	2.3	27		23	8.2	31		4.8	4.6	5.9
Benzo[e]pyrene	1.3	5.5	3.3	35	1.6	1.5	2.2	1.8		
Benzo[ghi]perylene	8.8			12	32	2.7	8.9	1.7	5.1	2.8
Dibenz[a,h]anthracene				310	3.2		2.4			
Coronene	7.8			7.1	730		3.2	1.9		
Indeno[1,2,3-cd]pyrene					7.4	1.9	7.0	1.8		
Anthanthrene	8.7							2.1		
Chrysene/Triphenylene	2.1	12	430	42	25	4.3	7.3	2.4	23	6.1
Benzofluoranthenes	5.7		2.0	28	7.1	5.0	3.4	1.7	7.7	120
Number of CRs over 50	0	2	2	1	2	0	0	0	1	1

C.O. = Coke Ovens
C.T. = Coal Tar
CFPP = Coal-fired Power Plants
C.S. = Coal Stoves
O&F = Open and Fireplace Burning
W.S. = Wood Stoves
D.E. = Diesel Emissions
G.E. = Gasoline Emissions
R.A. = Roofing Asphalt
P.A. = Paving Asphalt

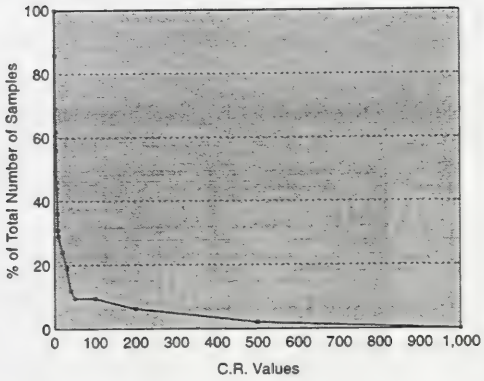


Figure 3.4.3.2.2.1.1.1 Distribution of confidence range values

Next, we pooled all confidence ranges regardless of mixture type or PAH involved. The results show that the CR is less than 50 for 90% of the samples and 6.0 or less for 50% of the samples (see figure 3.4.3.2.2.1.1.1). Thus for 90% of the samples, the mean relative PAH levels would fall within a narrow range that spans seven fold 95% of the time. The result indicates that the variation in PAH levels (relative to B[a]P) among samples from the same source mixture is relatively small. Even in the worst case scenario where the mean B[a]P levels differ by seven fold, the actual risk would differ by less than seven fold. In most instances, deviations from the mean would compensate each other, as some PAH levels would be higher than expected, while others are lower than expected.

It is apparent from table 3.4.3.2.2.1.1.1 that mixtures from different sources have approximately the same number of CRs over 50 (see bottom row of the table), and the number of CRs over 50 are not clustered around any particular PAH, as shown in table 3.4.3.2.2.1.1.2. We therefore conclude that the variation in PAH levels (relative to B[a]P) is minimal for all PAH across all mixtures listed in table 3.4.3.2.2.1.1.1.

Table 3.4.3.2.2.1.1.2 Number of confidence ranges exceeding 50 for individual PAH.

Compound	# of CRs ≥ 50
Anthracene	2
Phenanthrene	
Fluoranthene	
Pyrene	1
Benz[a]anthracene	2
Perylene	
Benzo[e]pyrene	
Benzo[a]pyrene	
Benzo[ghi]perylene	
Dibenz[a,h]anthracene	1
Coronene	1
Indeno[1,2,3-cd]pyrene	
Anthanthrene	
Chrysene/Triphenylene	1
Benzofluoranthenes	1

As a result of our findings, we establish the overall PAH profile for all source mixtures as the means of the relative levels of individual PAH of each mixture type. (see table 3.4.3.2.2.1.1.3 and appendix C).

B) PAH Profiles in Soils, Sediments and Ambient Air

In addition to source mixtures, we also examined environmental mixtures in different media. Our objective is to determine whether the PAH profile is similar in sediments, soils, and in ambient air. The analysis is presented in the exposure assessment document. Using the same criteria for source mixtures, we conclude that the profiles of PAH for sediment, soils and air samples are indeed similar.

Table 3.4.3.2.2.1.1.3 Mean profile of source mixtures and CRs for each PAH.

Compound	Mean	CR
Anthracene	3.9	9.7
Phenanthrene	18	2.2
Fluoranthene	4.9	2.7
Pyrene	4.5	5.2
Benz[a]anthracene	1.8	3.3
Perylene	0.51	3.5
Benzo[e]pyrene	1.0	2.4
Benzo[ghi]perylene	0.98	10
Dibenz[a,h]anthracene	0.35	14
Coronene	0.35	3.2
Indeno[1,2,3-cd]pyrene	0.51	7.46
Anthanthrene	0.49	2.7
Chrysene/Triphenylene	2.3	5.9
Benzofluoranthenes	1.6	2.5

C) PAH Profile for All Mixtures

This section compares the *typical profiles* of PAH of three environmental media (soil, sediment and ambient air) established in the exposure assessment document with the typical PAH profile of source mixtures. The purpose is to determine whether different environmental mixtures have a sufficiently similar composition so that they can be treated as identical for the purpose of risk assessment.

The typical profiles of mixtures in different environmental media have been summarized in sections 3.3.3.1 and 3.3.3.2. We conducted the analysis with the same approach that was used to evaluate the stability of source mixtures. The results are presented in tables 3.4.3.2.2.1.1.4 to 3.4.3.2.2.1.1.6.

Table 3.4.3.2.2.1.1.4 Confidence range analysis for comparing PAH profiles across media: whole air[a] (particulate and volatile component), soils, sediments and source mixtures.

Compound	Mean	Upper 0.95	Lower 0.95	CR
Anthracene	2.1	10	0.42	24
Phenanthrene	9.7	105	0.89	120*
Fluoranthene	5.8	20	1.7	12
Pyrene	4.1	14	1.1	13
Benz[a]anthracene	1.2	2.0	0.76	2.6
Perylene	0.42	1.9	0.093	20
Benzo[e]pyrene	0.97	2.2	0.42	5.3
Benzo[ghi]perylene	1.1	1.6	0.75	2.2
Dibenz[a,h]anthracene	0.28	0.57	0.13	4.2
Coronene	0.40	-	-	-
Indeno[1,2,3-cd]pyrene	0.86	1.9	0.40	4.7
Anthanthrene	0.25	1.1	0.060	18
Chrysene/Triphenylene	2.0	3.7	1.1	3.4
Benzofluoranthenes	2.1	4.1	1.1	3.9
Acenaphthylene	0.79	21	0.030	690*
Acenaphthene	1.3	21	0.078	260*
Fluorene	3.9	75	0.20	370*

[a] - Air data from Dann, 1989

* - Exceeds confidence range of 50

Table 3.4.3.2.2.1.1.4 shows that for most of the PAH considered, the CR values fall below the cut-off value of 50 across all media. These results indicate that, for risk assessment purposes, the profiles of PAH can be considered stable across the four media, and the variation in the levels of PAH relative to B[a]P is unlikely to be large enough to affect risk estimation.

The CR values exceed the cut-off value of 50 for four PAH (phenanthrene, acenaphthylene, acenaphthene and fluorene), suggesting that the profiles of the four PAH should not be considered stable across the four media. Examination of the data shows that the mean ambient air values for the four compounds are significantly higher than those reported for other media (see table 3.3.3.2.1). This behaviour is probably because all the four PAH have lower molecular weight, are more volatile and are less likely adsorbed on the particulates (Dann, 1989).

The ambient air data in table 3.4.3.2.2.1.1.4 are for whole air which includes both the particulates and the volatiles. In contrast, sediment and soil data represent PAH that are bound to the particulates only. As a result, whole air data are expected to have higher levels of volatile PAH than other data. Particulate-bound PAH, on the other hand, are expected to have similar profiles in the whole air and in soils and sediments.

In order to test this hypothesis, we examined if the stability of the profile for the four lower molecular weight PAH increased when only the air particulates, rather than the whole air, were considered. The annual air levels summary data for a number of PAH from various locations throughout Ontario (G. Diamond, private communication) were used for the analysis. Mean levels (relative to B[a]P) were determined for both the particulate-bound portion and the total air samples for each of the PAH listed (see table 3.3.3.2.2). The confidence range analysis for both of these data sets are shown in tables 3.4.3.2.2.1.1.5 and 3.4.3.2.2.1.1.6. The data clearly show that when only the particulate fraction of an air sample is considered, the PAH profiles across the four media can be considered similar (see table 3.4.3.2.2.1.1.5). However, when the whole air sample (particulate and air) is considered, the mixtures do not share similar profiles for the low molecular weight PAH (see table 3.4.3.2.2.1.1.6). The CR values for the more volatile, lower molecular weight PAH exceed the cut-off value of 50.

In conclusion, for the higher molecular weight PAH, the profiles can be considered similar in all media. The profiles for the lower molecular weight PAH can be considered stable only if the particulate fraction of an air sample is used to determine the profile.

Hence, we define the profile in table 3.4.3.2.2.1.1.5 the *Mixture of Standard Composition* (MSC). The MSC establishes the PAH profile (PAH level relative to B[a]P) of both source and environmental mixtures for a selected number of PAH.

Table 3.4.3.2.2.1.1.5 Confidence range analysis for comparing PAH profiles across media: air particulates[a], soils, sediments and source mixtures. **Column #2 in this table defines the *Mixture of Standard Composition (MSC)*.**

Compound	Mean	Upper 0.95	Lower 0.95	CR
Anthracene	1.6	8.0	0.33	24
Phenanthrene	4.3	20	0.89	23
Fluoranthene	3.8	5.1	2.8	1.8
Pyrene	2.8	4.8	1.7	2.9
Benz[a]anthracene	1.2	2.0	0.78	2.6
Perylene	0.45	1.9	0.11	17
Benzo[e]pyrene	1.1	2.9	0.39	7.4
Benzo[ghi]perylene	1.0	1.2	0.88	1.4
Dibenz[a,h]anthracene	0.28	0.57	0.13	4.2
Coronene	0.34	0.68	0.17	3.9
Indeno[1,2,3-cd]pyrene	0.86	1.9	0.38	4.7
Anthanthrene	0.31	-	-	-
Chrysene/Triphenylene	2.0	3.6	1.1	3.3
Benzofluoranthenes	2.5	8.1	0.76	11
Acenaphthylene	0.36	0.42	0.31	1.4
Acenaphthene	0.71	1.0	0.50	2.0
Fluorene	1.6	11	0.23	47

[a] - Air data from Diamond, private communication

* - Exceeds confidence range of 50

Table 3.4.3.2.2.1.1.6 Confidence range analysis for comparing PAH profiles across media: whole air[a] (particulate and volatile component), soils, sediments, and source mixtures.

Compound	Mean	Upper 0.95	Lower 0.95	CR
Anthracene	1.7	7.9	0.39	20
Phenanthrene	7.7	38	1.5	25
Fluoranthene	4.8	9.9	2.3	4.3
Pyrene	3.4	7.3	1.6	4.5
Benz[a]anthracene	1.3	1.8	0.92	1.9
Perylene	0.63	1.9	0.21	9.2
Benzo[e]pyrene	1.1	2.1	0.57	3.7
Benzo[ghi]perylene	0.96	1.0	0.92	1.1
Dibenz[a,h]anthracene	0.28	0.41	0.19	2.1
Coronene	0.41	0.59	0.29	2.1
Indeno[1,2,3-cd]pyrene	0.86	1.4	0.53	2.7
Anthanthrene	0.23	0.51	0.11	4.8
Chrysene/Triphenylene	1.7	2.7	1.1	2.4
Benzofluoranthenes	2.3	4.1	1.3	3.2
Acenaphthylene	0.85	13	0.056	230
Acenaphthene	1.6	22	0.11	200
Fluorene	3.3	31	0.36	85

[a] - Air data from Diamond, private communication.

* - Exceeds confidence range of 50.

3.4.3.2.2.1.2 Conclusion

Based on the analysis summarized above, we conclude that a wide number of source mixtures have a fairly similar profile of commonly assayed PAH. We

therefore expect that the PAH fraction of each source or environmental mixture has a similar PAH profile and that this profile will be similar to the *Mixture of Standard Composition (MSC)* as defined in table 3.4.3.2.2.1.1.5.

The conclusion does not apply to substituted PAH, as there is strong evidence indicating that different mixtures have different levels of substituted PAH compounds. The conclusion does not imply that there are no real differences between mixtures dependent on the source of the mixture, fuel type and the pyrolysis conditions that produce the emission mixture. Furthermore, air transport, degradation in sunlight or by soil microorganisms, and other factors may alter the PAH profile in the environment. However, these factors do not generate large enough difference in the PAH profiles that would significantly alter the human health risk due to exposure to a given mixture.

3.4.3.2.2.2 Potency of Mixtures

If B[a]P were a suitable indicator for the potency of a mixture, then the potency of the mixture expressed as tumor incidence per ng of B[a]P content should give numerically similar answers for all mixtures where PAH are expected to be the major cause of cancer effects. We tested this hypothesis using the method described in section 3.4.3.2.1 and found that, in general, all different types of mixtures were roughly equipotent (potency expressed in terms of B[a]P content) in the tumor initiation assay (see table 3.4.3.2.2.2.1). The difference was larger when organic extract was used as the indicator. With B[a]P as the indicator, the maximum/minimum ratio was lowered by four fold and the standard deviation/mean ratio was lowered by about three fold. Similarly, the risk estimates from the three risk assessments based on human data using the WHO type method are also very similar (see table 3.3.1.1).

3.4.3.2.3 Conclusion - Selection of Indicator

We decide to select B[a]P to serve as an indicator for the PAH fraction of complex mixtures. Our decision is supported by the following observations.

- Organic extract has been shown to be an inferior indicator for the potency of similar mixtures.
- B[a]P is a reasonable indicator for the levels of a number of unsubstituted PAH in the mixture.
- Different PAH-rich mixtures with the same B[a]P content have approximately the same potency.

In order to use this risk assessment model, one needs to measure the ambient levels of B[a]P in a given environmental medium. The present document develops the relationship between the airborne B[a]P levels and human risk. Using the two pieces of information, one can estimate the risk attributable to the PAH component of the mixture. The model is referred to as the *Whole Mixture Model.*

Table 3.4.3.2.2.2.1 Potency of various PAH-containing mixtures in tumor initiation assay. Results are calculated from the data of Nesnow *et al.* (1982).

Mixture	Incidence/mg of organics	Incidence/ng of B[a]P
Coke oven main	170	9.4 E-2
Coke oven topside	130	7.6 E-2
Smoky coal	66	7.8 E-2
Smokeless coal	33	2.8 E-2
Roofing tar	9.6	1.1 E-2
Wood Smoke	12	5.8 E-2
Diesel engine exhaust	15	2.5 E-2*
Gasoline engine exhaust	5.8	5.6 E-2
Oil burning residential furnace	1.6	NA
SD/mean	1.1*	0.55
Max/min	29*	8.6

* - Excludes the data for oil burning furnaces

3.4.3.3 Selection of Alternatives for Risk Assessment

The present document conducts risk assessment for PAH using both the *Individual PAH Model* (IPM) and the *Whole Mixture Model* (WMM) because both models have important and complementary advantages. IPM is more compatible with the approaches taken by other jurisdictions and the traditional approach of assessing health risk posed by mixtures. The PAH compounds considered in the assessment are explicitly defined. Because IPM considers only a small proportion of PAH that are present in the mixture, the approach significantly underestimates the risk posed by the PAH fraction (Thorslund and Farrar, 1990).

The WMM model is used much less often in North America, although it is rather popular in Europe (WHO, 1987; Slooff *et al.* 1989). It is a simple method which accounts for the toxicity of non-substituted PAH as a whole. Its main disadvantage stems from it's dependence on the assumption that PAH potency is proportional to the B[a]P content. Although we have provided evidence that this assumption holds in some instances, it is not possible to prove that it holds in all situations where the method is applied. It is expected that the WMM will, on average, slightly overestimate the risk due to PAH in mixtures because the lung cancer risk to coke oven emissions is attributable entirely to the PAH fraction. The overestimation may be small since Thorslund and Farrar (1990) found that, for a number of PAH-rich mixtures, the bulk of their carcinogenicity is attributable to the fraction of the mixture containing PAH with 4-7 rings.

3.4.4 Species-to-Species Extrapolation Based on Surface Area or Body Weight

Very little human data are available to allow determination of human cancer risk due to individual PAH and to most mixtures, so the potency must be extrapolated from experimental animals. Moreover, the experimental data strongly suggest that the potency and the site of action of PAH are dependent on the route of administration (see sections 3.4.8 and 3.4.9). It is therefore likely that route-specific differences exist in humans as well, making it necessary to develop route-specific estimates for the potencies of B[a]P and other PAH.

The most common strategy to extrapolate animal data to humans is to base the extrapolation on the relative surface areas or body weights of the species. This strategy assumes that the relative body weight or surface area is a good predictor of the relative potency of PAH in the two species. While such scaling factors have been shown to hold for other compounds (see Chappell, 1989 for references) and have previously been used for PAH (Thorslund and Farrar, 1990; Collins and Alexeeff, 1993; Collins *et al.* 1991), the relationship has not been validated for PAH.

We intend to examine if surface area or body weight extrapolation is a suitable method to conduct animal-to-human extrapolation for PAH. No data are available to allow direct comparison of the response between humans and animals, but different rodent strains have been tested with similar assays. If the surface area and/or body weight extrapolations were valid for rodent-to-human extrapolation, the same extrapolation should hold for closely related rodents. Therefore, we compared the actual dose to the *extrapolated* dose that is expected to produce the same level of tumorigenic response in the same target rodent species. The *extrapolated dose* in the target species was estimated from the dose required to induce the same level of response in the reference rodent species under matching experimental conditions using a scaling factor based on the relative body weight or surface area.

The surface area and body weight extrapolation methods assume that the potency of PAH is a function of the body size and that there are no qualitative difference in how the different species metabolize and respond to PAH. Therefore, we also examined the metabolic fate and pharmacokinetics of PAH in different mammalian species to evaluate if the extrapolation method based on body weight or surface area is appropriate for predicting PAH toxicity in humans from animal data.

3.4.4.1 Tumor Response

3.4.4.1.1 Methods

We examined the tumorigenicity potency of PAH in different experimental animal species using the B[a]P and 3-MC data. The data used in the analysis were selected from the data reported in Appendix A.

The assessment was conducted as follows.

- The most compatible studies were selected for comparison. Factors like route of administration, test compound, and treatment regimen were kept the same or similar. The only outstanding difference in the experimental design between studies that were compared was the animal species involved.
- Next, the tumorigenic potency for B[a]P and 3-MC was calculated and expressed as incidence per mg of B[a]P and 3-MC, respectively.
- Finally, the potencies of PAH associated with different species were compared for both B[a]P and 3-MC.

The selection of data for comparing the tumorigenicity potency for each route of exposure and each PAH was governed by the following guidelines.

- The experiments selected for comparison have to be compatible to the greatest extent possible.
- Preference is given to studies where 3-MC or B[a]P is administered repeatedly or in a form that allows sustained release.
- A study where PAH is administered in a single dose would not be compared to another study where PAH is administered in multiple doses. A single dose of PAH tends to induce a larger effect than if the same dose is administered in small portions over an extended period of time (see Section 3.8.2).
- A study where PAH is administered by implantation would not be compared to another study where PAH is administered by intratracheal instillation. Implantation of PAH induces a larger tumorigenic response than when PAH is administered by instillation (see section 3.3.2.1.3).

- The potency is determined for the tumors and sites where tumors are induced at the lowest PAH dose administered.

Once the above constraints were met, we selected the studies that yielded the biggest response in terms of incidence/mg for each species and for each PAH. Then for each PAH and each route of exposure, we estimated the dose expected to illicit the same level of tumor response in the target species as observed by extrapolation from the reference species. Extrapolation based on both body weight and surface area were conducted using equation 3.4.4.1.1.1 to equation 3.4.4.1.1.3. Equation 3.4.4.1.1.1 calculates the extrapolated incidence per mg PAH predicted for the target species based on **body weight** (b.w.) Extrapolation, while equation 3.4.4.1.1.2 calculates the extrapolated incidence per mg PAH based on **surface area** extrapolation. Equation 3.4.4.1.1.3 calculates the extrapolated dose expected to induce the same level of tumor response in the target species as observed.

$$\frac{incidence}{mg \quad PAH_{target}} = \frac{incidence}{mg \quad PAH_{ref}} \times \frac{b.w._{ref}}{b.w._{target}}$$ Equation 3.4.4.1.1.1

$$\frac{incidence}{mg \quad PAH_{target}} = \frac{incidence}{mg \quad PAH_{ref}} \times \left\{ \frac{b.w._{ref}}{b.w._{target}} \right\}^{2/3}$$ Equation 3.4.4.1.1.2

Assumed weights:

Mouse.....	30 g
Rat.....	300 g
Hamster.....	100 g
Monkey.....	10 kg

$$extrapolated \quad dose_{target} = \frac{observed \quad incidence_{target}}{incidence/mg_{PAH,target}}$$ Equation 3.4.4.1.1.3

The actual dose in the *target species* was then compared to the extrapolated dose as predicted from the *reference species*. The *actual dose* is the dose the target species were exposed to in the experiment. The *extrapolated dose* is the dose estimated from the reference species data by surface area or body weight extrapolation, and is expected to produce the same level of response in the target species as the actual dose. If the tested dose elicited no tumors in the target species, an assumption of 1% incidence for the actual dose was used in the comparison to the extrapolated dose.

The results are presented in section 3.4.4.1.2.

3.4.4.1.2 Results

3.4.4.1.2.1 Rodent Studies

Section 3.4.4.1.2.1 presents the results of the comparison between the actual doses and extrapolated doses (based on extrapolation from mouse data) that would achieve the same level of tumor incidence in rats and hamsters. The results are summarized in Tables 3.4.4.1.2.1.1 to 3.4.4.1.2.1.3. The ratio of extrapolated dose to actual dose serves as an indicator for the predictability of the extrapolation method.

Oral Exposure

Table 3.4.4.1.2.1.1 compares the actual dose to the extrapolated dose that is expected to induce the same level of incidence of forestomach tumor in two animal species by oral exposure. Predictions based on either body weight or surface area extrapolation from the mouse data invariably overestimate the sensitivity of both the rat and the hamster. The difference between the extrapolated and the actual doses is within one order of magnitude in the hamster and one to two orders of magnitude in the rat.

The difference is generally less pronounced when B[a]P rather than 3-MC was used as the test substance. The extrapolated dose differs from the actual dose by one to two orders of magnitude when the animals were administered 3-MC and by an order of magnitude when the test substance was B[a]P.

Respiratory Exposure

The results presented in table 3.4.4.1.2.1.2 indicate that rats are more sensitive to respiratory exposure to 3-MC or B[a]P than predicted from the mouse data. The difference between the actual and extrapolated dose is within one to two orders of magnitude. In the case of hamsters, the sensitivity is predicted with reasonable reliability when B[a]P was administered to the animals and somewhat underestimated when the test substance was 3-MC.

Table 3.4.4.1.2.1.1 Comparison of actual and extrapolated doses of 3-MC or B[a]P expected to induce the same level of tumor incidence as observed in the target species. Oral administration and forestomach tumors.

Target Species	Compound	Actual Dose	Extrapolated Dose		Extrapolated/ Actual Dose		Reference
			Sur. Area	Body Wt	Sur. Area	Body Wt	
rat	3-MC	960 mg*	5.2 mg	11.4 mg	0.054	0.012	Lorenz & Stewart, 1948; Shay *et al.* 1949
hamster	3-MC	250 mg	28 mg	42 mg	0.11	0.17	Lorenz & Stewart, 1948; Homburger *et al.* 1972
rat	B[a]P	14 mg**	3.8 mg	8.5 mg	0.27	0.61	Wattenberg *et al.* 1979; Brune *et al.* 1989
hamster	B[a]P	46 mg***	31 mg	46 mg	0.67	1.0	Wattenberg *et al.* 1979; Dontenwill & Mohr, 1962

* - The tumor incidence was 0 % at a dose of 960 mg. For the purpose of the present exercise, 1% tumor incidence was assumed. In reality, the dose required to achieve 1% forestomach tumor incidence could be much higher than 960 mg. Even though no gastric tumors were reported, 960 mg induced a high incidence of breast adenocarcinoma (50-70%).

** - Author also conducted gavage experiments with 1.5% caffeine in the test substance. We have not included these studies in the analysis because of possible interactions between the effects of B[a]P and caffeine.

*** Papillomas considered

Table 3.4.4.1.2.1.2 Comparison of actual and extrapolated doses of 3-MC or B[a]P expected to induce the same level of tumor incidence as observed in the target species. Respiratory exposure and lung tumors.

Target Species	Compound	Actual Dose	Extrapolated Dose		Extrapolated/ Actual Dose		Reference
			Sur. Area	Body Wt	Sur. Area	Body Wt	
rat	3-MC	0.50 mg*	7.0 mg*	15 mg*	14*	30*	Nettesheim & Hammons, 1971; Hirano *et al.* 1974
hamster	3-MC	0.7 mg*	4.7 mg*	7.1 mg*	6.7*	10*	Nettesheim & Hammons, 1971; Hammond *et al.* 1987
rat	B[a]P	15 mg	31 mg	33 mg	2.1	2.2	Furst *et al.* 1979; Ishinishi *et al.* 1979
hamster	B[a]P	30 mg	33 mg	222 mg	1.1	0.73	Furst *et al.* 1979; Saffiotti *et al.* 1972

* - Nettesheim and Hammons (1971) study utilized intratracheal instillation, while Hirano *et al.* (1974) used intralung pellets to administer 3-MC to rats. Hammond *et al.* (1987) applied intrabronchial pellets to hamsters. As indicated in section 3.3.2.1.3, instillation is less effective in inducing tumors than implantation. This is a likely explanation for the apparent discrepancy between the actual and extrapolated doses.

Table 3.4.4.1.2.1.3 Comparison of actual and extrapolated doses of 3-MC or B[a]P expected to induce the same level of tumor incidence as observed in the target species. Topical administration and skin tumors.

Target Species	Compound	Actual Dose	Extrapolated Dose		Extrapolated/ Actual Dose		Reference
			Sur. Area	Body Wt	Sur. Area	Body Wt	
rat	3-MC	58 mg	9.0 mg	20 mg	0.16	0.35	Cavalieri *et al.* 1978; Zackheim, 1964
hamster	3-MC	5.0 mg*	0.048 mg	0.074 mg	0.0096	0.015	Cavalieri *et al.* 1978; Bernfeld & Homburger, 1983
rat	B[a]P	N.A.					N.A.
hamster	B[a]P	5.0 mg*	0.019 mg	0.028 mg	0.0038	0.056	Cavalieri *et al.* 1983; Bernfeld & Homburger, 1983

* - No PAH-induced tumors at tested dose. Assumed 1% response rate.

Dermal Exposure

The interpretation for dermal exposure is confounded by the paucity of locating good studies using the rat skin model. Zackheim (1964) did not report the experimental results separately for the different strains of rats, for the different durations of treatment, or even for the two different dosing regimens used in the study. The author claimed that the experimental results did not differ significantly when animals were dosed using two different dosing regimens[15]. These shortcomings in the experimental design limit the reliability of the comparison between the potency of PAH in inducing skin tumors in mice and rats.

Body weight and surface area extrapolations from mouse data appear to overestimate the sensitivity of both rats and hamsters to the action of PAH, but especially hamsters (see table 3.4.4.1.2.1.3). The type of response also seems to differ in hamsters. Mice and rats developed squamous cell carcinomas after treatment with PAH. Hamsters, on the other hand, developed skin melanomas with DMBA treatment, and no tumor was observed after treatment with B[a]P or 3-MC (Bernfeld and Homburger, 1983). Often a single dose of DMBA was sufficient to induce tumors in hamsters and no promotion was required. In contrast, promotion was generally required to induce a high tumor incidence in mice.

3.4.4.1.2.2 Monkey Studies

We also examine whether surface area and body weight extrapolations hold for mammals other than rodents. Monkeys seem to be an ideal species for the analysis as they are more phylogenetically related to humans than rodents. Unfortunately, there are very little data on monkeys and the available data do not lend themselves readily to the analysis.

The study of Adamson and Sieber (1983) is not the ideal study for our purpose because of the use of diverse treatment schedules for different monkeys and the small number of animals involved in the study, as well as the lack of experimental details in the report. The investigators used a mixed group of "Old World monkeys" of various strains, including rhesus, cynomolgus and African green, in their study. The animals were given either subcutaneous injections of 10 to 40 mg/kg of 3-MC for a total of 1 to

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15 3-MC-treated rats (14 rats) received 0.18 ml of a 0.3 % solution 3 times weekly for 180 days. The rest of the rats (about 28) were treated for 250 to 300 days. We assume that all 42 rats were treated for 250 days in calculating the total dose.

12 doses (7 monkeys), or they were given 20 to 120 mg/kg of 3-MC orally 5 to 7 days a week for 5 years (14 monkeys). Animals were under observation for up to 15 years. No tumors were detected at necropsy or upon histopathologic examination of the animals that died by the time the report was being prepared (eight of fourteen that were given PAH orally and five out of seven receiving s.c. administration).

We made use of the oral exposure data only in our assessment because humans are not normally exposed to PAH subcutaneously. We assumed that all "Old world monkeys" in Adamson and Sieber study were rhesus monkeys weighing 10 kg (Bourne, 1975) and that the monkeys received 70 mg of 3-MC/kg 6 days per week for 5 years. Since Adamson and Siebert did not report any tumors in their study, the dose required to induce submaximal, but significant tumor rates (such as those seen in the rodents) would have to be considerably higher than the administered dose. We assumed a tumor incidence rate of 1% in monkeys in our analysis. Although the study is not ideal for reliable numerical analysis, we believe that if the treatment had any significant effects, the effects would have been observed despite the small number of monkeys in the study and other experimental shortcomings.

The present exercise predicts a dose that would induce the same level of tumor response in the monkeys based on rodent data. The actual and extrapolated doses were then compared in a similar fashion as for the rodent species. Results are shown in table 3.4.4.1.2.2.1. The results indicate that the extrapolated doses expected to induce marginal tumor rates in monkeys are orders of magnitude lower than the actual doses which were administered to the animals and which did not induce a tumorigenic response. Thus the extrapolation methods overestimate the potency of 3-MC in monkeys by about two and a half to four orders of magnitude.

The differences between the predicted and actual doses are very large, and it is unlikely that inconsistency in experimental methodologies can fully account for these differences. Furthermore, negative tumor response has been reported for rhesus monkeys after being exposed subcutaneously or dermally to B[a]P (Pfeiffer and Allen, 1948, cited in Osborne and Crosby, 1987). In addition, cases of human exposure to B[a]P have been discussed in the literature (Cook and Kennaway, 1940; Cottini and Mazzone, 1939; both cited in Osborne and Crosby, 1987), but none of them reported any tumors.

Table 3.4.4.1.2.2.1 Comparison of actual and extrapolated doses of 3-MC expected to induce the same level of tumor incidence in monkeys. Oral administration in rodents and monkeys.

Reference Species	Actual Dose	Extrapolated Dose		Extrapolated/ Actual Dose		Reference
		Sur. Area	Body Wt	Sur. Area	Body Wt	
mouse	abt. 1100 g*	0.053 g	0.39 g	2.1 E4	2.8 E3	Adamson & Sieber, 1983; Lorenz & Stewart, 1948
rat	abt. 1100 g*	0.067g**	0.22 g**	1.6 E4**	5.0 E3**	Adamson & Sieber, 1983; Shay *et al.* 1949
hamster	abt. 1100 g*	0.48 g	2.2 g	2.3 E3	5.0 E2	Adamson & Sieber, 1983; Homburger *et al.* 1972

* No 3-MC-induced tumors at tested dose in monkeys. Assumed 1% response rate.
** The only tumors observed were breast tumors in female rats. Since the gender of the monkeys was not reported and since breast tumors develop under normal circumstances only in female rats, it is not certain whether the results from the rat study can be used for comparison with the monkey study.

3.4.4.2 Pharmacokinetics

The metabolism of PAH is reviewed in appendix D and conclusions are drawn based on the data presented in the appendix. The biochemical basis for species difference is unclear. However, it appears that cytochrome P-450c (or AHH) is more abundant in mouse liver and lung tissues than in the homologous rat tissues (see appendix D). Since this enzyme is responsible for the formation of both primary epoxides and diol epoxides, the putative proximal carcinogens, the results would suggest that mice may be more sensitive to PAH than rats. Furthermore, the levels of enzymes that can remove the reactive compounds, such as epoxide hydrolase (EH) and glutathione-S-transferase (GST), relative to AHH levels, could have an effect on the availability of the reactive metabolites for binding to DNA. EH catalyzes the conversion of epoxides to diols, the precursors of the more reactive diol epoxides. Although the enzyme levels of GST relative to AHH (GST/AHH) are similar in the rat and mouse tissues, the EH/AHH ratio is lower in the mouse liver than in the rat liver (similar in the lungs, refer to appendix D). This observation implies that the diol epoxides formed by AHH activity are removed more slowly in the mouse than in the rat tissues. The resulting higher potential for DNA adduct formation in the mouse explains in part its higher sensitivity to the tumor induction properties of PAH.

Rats, mice and hamsters all form 4,5-, 7,8- and 9,10-B[a]P diols, the precursors of the putative proximal carcinogens, diol epoxides. However, the relative content of the three diols appears to be different in different species (see appendix D and table 3.4.4.2.1). The 7,8-diol predominates (54% of all diols formed) in mice, but not in rats or hamsters. Only 7,8-diol can be metabolized into the most reactive epoxide, 7,8-diol epoxide. In addition, only the (-) B[a]P-7,8-diol is converted to (+) 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, the most active B[a]P-diol epoxide. Without knowing whether any difference exists in the (+) B[a]P-7,8-diol/(-) B[a]P-7,8-diol ratio across species, it is difficult to correlate the difference in metabolite profiles with the difference in species sensitivity to PAH.

The DNA adduct profiles that have been identified in mice and rats after topical exposure are very similar. In both instances, most of the DNA adducts are due to a reaction of the 7,8-diol epoxide with DNA. Other minor DNA adducts, such as 9-hydroxy-DNA and 4,5-epoxy-DNA, are also present (4,5-epoxy-DNA is not found in the mouse), but they constitute a very small proportion (< 20%) of the total adducts. These results are consistent with the assumption that the B[a]P-7,8-diol epoxide reacts with mouse and rat DNA in a similar manner and that it is primarily this diol epoxide that gives rise to the main bulk of the DNA adducts in both species.

There are not enough data to determine the extent and significance of the difference in PAH metabolism to species sensitivity. Nevertheless, the available data suggest that there may

be metabolic differences between species that affect the degree to which the species are vulnerable to the toxic effects of PAH. The differences may very well not be a simple function of body size, surface area or basal metabolic activity.

Table 3.4.4.2.1 Relative content of three B[a]P diols in the rat, the mouse and the hamster.

Diol	% of All Three Diols		
	Rat	Mouse	Hamster
B[a]P-4,5 diol (±)	18	13	74
B[a]P-7,8 diol (±)	27	54	12
B[a]P-9,10 diol (±)	55	33	14

3.4.4.3 Discussion and Conclusion

Many of the problems encountered in the evaluation of species-species extrapolation methods based on body weight or surface area are due to the heterogeneity of the data sets used in the analysis. The problems have been described in section 3.3.2.2.2 and are common to all other analyses using the same sets of data.

Mice and rats are closely related rodent species of similar sizes. They have similar diets and are housed in a similar manner in the laboratory. One would expect that the extrapolation methods would have the best chance of success when extrapolation is conducted from one rodent species to another. However, the extrapolated dose predicted to induce the same level of tumor response as observed in the target species differed from the actual administered dose by as much as two orders of magnitude when both the target and reference species were rodents. When the methods were used to extrapolate from rodents to monkeys, the discrepancy was even bigger. Although some of the discrepancies may be due to methodological problems, the major cause is likely the low predictive capability of the two extrapolation methods. Therefore, it is possible that extrapolation from rodents to humans based on body weight or surface area may lead to large errors.

Species-to-species extrapolations based on surface area or body weight assume that species difference in the metabolism of the parent compound to the primary carcinogen is a function of the body weight or surface area. The available pharmacokinetic data do not support this assumption in the case of PAH.

Finally, the studies used in PAH risk assessments sometimes involve direct deposit of PAH onto or in close proximity to the tissues where tumors or carcinomas are later observed.

Since the dose delivered to the tissues is direct and is not a reflection of the body weight or body surface, body weight or surface area species-to-species extrapolations may not be appropriate for PAH.

In conclusion, estimation of PAH potency in humans based on surface area or body weight extrapolation from rodent data could likely lead to very substantial errors. Other approaches, such as relative potency approach, would be preferable for the estimation of human risk from animal data.

3.4.5 Extrapolation Using Physiologically Based Pharmacokinetic Models

An alternative strategy to body weight and surface area extrapolations is to use physiologically based pharmacokinetic (PB-PK) models (refer to Menzel, 1987; Lutz and Dedrick, 1987; Park, 1989 about PB-PK models). These models estimate the relationship between the administered dose and the dose delivered to a particular tissue. The relationship is species specific. Extrapolation using PB-PK models assumes that the toxic response results from tissue exposure to toxic chemicals and that a particular tissue dose induces the same intensity of response in all mammalian species including humans. The species-specific relationship between the administered dose and tissue dose will determine the difference in the sensitivity of the different species to PAH.

Use of PB-PK models in conducting extrapolations in cancer risk assessment requires full understanding of the mechanism of action of the toxicants and the kinetics of the formation and removal of the putative carcinogenic metabolites in the body. While data are available for some of the parameters needed in model development, PB-PK models suitable for PAH risk assessment are not readily available.

3.4.6 Relative Potency Approach to Extrapolation

A third approach to extrapolation is called the **relative potency approach** (Albert *et al.* 1983; Thorslund and Charnley, 1988; Krewski *et al.* 1989). This method is relatively simple and depends on one key testable assumption. The assumption maintains that the relative potency of PAH and of PAH-containing complex mixtures is independent of species, strains and the route of administration. The assumption can be restated as "the relative potency of a particular PAH or PAH-containing complex mixture to another PAH or mixture is the same in all mammalian species" (see equation 3.4.6.1a), or "the relative sensitivity of different species to a given PAH is the same for all carcinogenic PAH" (see equation 3.4.6.1b).

$$C_a = \frac{human\ \ risk\ \ (PAH\ \ 1)}{human\ \ risk\ \ (PAH\ \ 2)} = \frac{exper.\ \ potency\ \ (PAH\ \ 1)}{exper.\ \ potency\ \ (PAH\ \ 2)}$$ Equation 3.4.6.1a

$$C_b = \frac{human\ risk\ (PAH\ 1)}{exper.\ potency\ (PAH\ 1)} = \frac{human\ risk\ (PAH\ 2)}{exper.\ potency\ (PAH\ 2)} = \frac{human\ risk\ (mix.1)}{exper.\ potency\ (mix.1)}$$ Equation 3.4.6.1b

We select the relative potency approach for extrapolation because of the relative simplicity of the approach, and because good data sets are available for extensive validation of the underlying assumptions. This section presents evidence in support of the suitability of the relative potency approach for extrapolation purposes. Our key arguments are summarized below.

- We confirmed previous reports (Slaga and Fisher, 1983) that the relative potency of individual PAH to B[a]P is very similar in different strains of mice and in different mouse tumor initiation models (see section 3.4.6.1). Therefore, the potency of a given PAH in one assay can be predicted from the relative potency of two PAH in another mouse skin assay if the potency of the second PAH is known in the first assay.
- We showed that the relative potency of 3-MC to B[a]P holds across different species and routes of administration (see table 3.4.6.2.2.1). Furthermore, we demonstrated that there is a good correlation between the relative potency of individual PAH in mouse skin tumor initiation assays and rat lung assays (see section 3.4.6.2.1). Since the relative potency of PAH in mouse skin and rat lungs appears to be the same, the relative potency of PAH is expected to be similar between mouse skin and human lungs and other human tissues, regardless of the route of exposure.
- We confirmed (see Albert *et al.* 1983; Lewtas, 1985, 1985b; Nesnow, 1989, 1990) that the relative potency of several source mixtures in humans and in experimental animals is comparable even when the route of exposure is different (see section 3.4.6.3). This suggests that the relative potency approach also applies to mixtures.
- We demonstrated that the relative potency of mixtures to B[a]P is similar in mouse skin and rat lung models, suggesting that the relative potency also applies to a combination of mixtures and individual compounds (see section 3.4.6.4).

The basis for the above arguments is presented in the following subsections.

3.4.6.1 Relative Potency of PAH in Different Mouse Strains

Slaga and his coworkers (1982) compared the relative potency of DMBA and B[a]P in SENCAR and CD-1 mice. They found that the relative potency of DMBA to B[a]P (defined as the ratio of tumor potency of DMBA to B[a]P) was more than an order of magnitude higher in the SENCAR mice than in CD-1 mice in the tumor initiation assay (see figure 3.4.6.1.1). The enhanced sensitivity of the SENCAR strain to DMBA relative to B[a]P may be due to selection for high DMBA response during the inbreeding of the SENCAR strain. However, DMBA was generally more potent than B[a]P in both mouse strains.

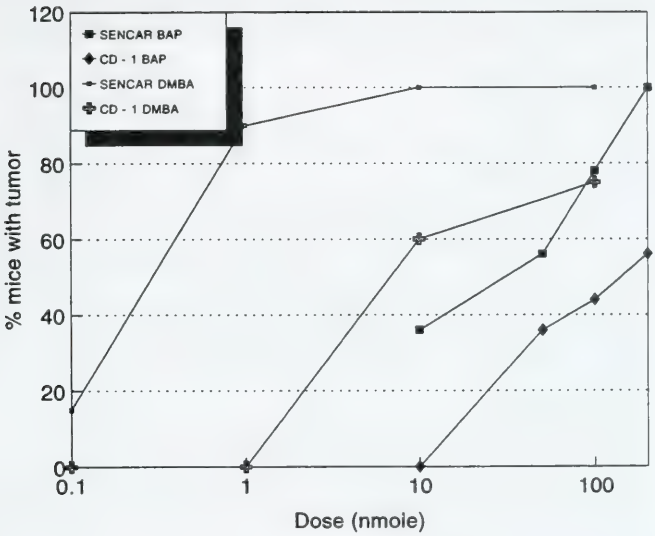

Figure 3.4.6.1.1 Relative sensitivity of mouse strains to B[a]P and DMBA. Data were extracted from Slaga et al. 1982.

We identified fifteen compounds for which we estimated and compared the tumor potency for both SENCAR and CD-1 mice (see section 3.5.6.2 for a description of the estimation of potency for these compounds). The potency estimates are shown in table 3.4.6.1.1. Note that figure 3.4.6.1.1 shows that the SENCAR mice are more sensitive to PAH than CD-1 mice, while table 3.4.6.1.1 implies the reverse. The disagreement is probably due to the difference in the dose of the promotor administered. In figure 3.4.6.1.1, both strains of mice received the same dose of a promotor (8.5 nmoles of TPA, twice a week). Under such conditions, SENCAR mice were clearly more sensitive to PAH. In contrast, the SENCAR mice in table 3.4.6.1.1 were promoted with only 3.2 nmoles of TPA twice a week, while the CD-1 mice received 16 nmoles of TPA biweekly. CD-1 mice appeared to be more sensitive, presumably because they were promoted with TPA at a dose five times higher than what was given to the SENCAR mice.

Table 3.4.6.1.1 Relative sensitivity of SENCAR and CD-1 mouse strains to PAH carcinogenicity.

Compound	Slope* CD-1 (incidence/μg)	Slope* SENCAR (incidence/μg)	SENCAR /CD-1
benz[a]anthracene, 7,12-dimethyl-,	107	31.743	0.296
cholanthrene, 3-methyl-,	14.043	3.179	0.226
benzo[a]pyrene	5.798	2.366	0.408
benzo[a]pyrene, 7,8-dihydroxy-7,8-dihydro-, trans-, (+/-)	2.653	1.240	0.467
dibenzo[a,h]anthracene	4.675	1.578	0.336
dibenz[a,h]anthracene, 3,4-dihydroxy-3,4-dihydro-, trans-, (+/-)	4.183	0.992	0.237
benzo[a]pyrene, 6-methyl-,	0.616	0.338	0.548
chrysene, 1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (+/-)	0.197	0.050	0.255
benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-7,12-dimethyl-, trans-, (+/-)	95.599	32.374	0.339
benz[a]anthracene, 8,9-dihydroxy-8,9-dihydro-7,12-dimethyl-, trans-, (+/-)	0.177	0.344	1.941
chrysene	0.166	0.138	0.833
cyclopenta[cd]pyrene	0.076	0.120	1.584
benz[a]anthracene, 5,6-dihydroxy-5,6-dihydro-7,12-dimethyl-, trans-, (+/-)	0	0	NA
benzo[e]pyrene	0	0	NA
benzo[e]pyrene, 9,10-dihydroxy-9,10-dihydro-, trans-, (+/-)	0	0.038	NA

* - estimated from linear regression.

Table 3.4.6.1.2 Correlation of PAH potency between SENCAR and CD-1 mouse strains. Potency estimates are presented in table 3.4.6.1.1.

Parameter	Parameter Value
Pearson, r	0.997
p-value	0.0001
N (# of data points)	15

Table 3.4.6.1.3 Relative sensitivity of CD-1 mice (tumor initiation) and C57BL mice (complete carcinogenesis) to PAH carcinogenicity.

Compound	Slope* CD-1 (incidence/μg)	Slope* C57BL (incidence/μg)	C57BL /CD-1
benz[a]anthracene, 7,12-dimethyl-,	0.107**	0.651	0.006
benzo[a]pyrene	5.798	0.146	0.025
benzo[a]pyrene, 7,8-dihydroxy-7,8-dihydro-, trans-, (+/-)	2.653	0.162	0.061
benzo[a]pyrene, 9,10-dihydroxy-9,10-dihydro-, trans-, (+/-)	0.066	0	0
benzo[a]pyrene, 7,8-dihydro-7,8-epoxy-, (+/-)	2.436	0.020	0.008
benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (+/-)	0.976	0.004	0.004
benzo[a]pyrene, 7α,8ß-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+/-)	0	0	NA
benzo[a]pyrene, 1-hydroxy-,	0	0	NA
benzo[a]pyrene, 2-hydroxy-,	0.759	0.209	0.275
benzo[a]pyrene, 3-hydroxy-,	0.133	0	0
benzo[a]pyrene, 4-hydroxy-,	0	0	NA
benzo[a]pyrene, 5-hydroxy-,	0.121	0	0
benzo[a]pyrene, 6-hydroxy-,	0.158	0	0
benzo[a]pyrene, 7-hydroxy-,	0.158	0	0
benzo[a]pyrene, 8-hydroxy-,	0	0	NA
benzo[a]pyrene, 9-hydroxy-,	0.279	0	0
benzo[a]pyrene, 10-hydroxy-,	0.121	0	0
benzo[a]pyrene, 11-hydroxy-,	0.708	0.004	0.006
benzo[a]pyrene, 12-hydroxy-,	0.279	0	0
benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-, (+/-)	0.395	0.001	0.003
benzo[a]pyrene, 9,10-dihydro-9,10-epoxy-, (+/-)	0.162	0	0
benzo[a]pyrene, 11,12-dihydro-11,12-epoxy-, (+/-)	0.590	0	0

* - estimated from linear regression.

** - DMBA potency (from CD-1 assay) expressed in incidence/ng.

Table 3.4.6.1.4 Correlation of PAH potency in C57BL (complete carcinogenesis) and CD-1 (tumor initiation) mouse strains. Potency estimates are presented in table 3.4.6.1.3.

Parameter	Parameter Value
Pearson, r	0.924
p-value	0.0001
N	22

We evaluated the Pearson correlation coefficient, r, between the potency of PAH in the two species for the fifteen PAH using the data in table 3.4.6.1.1. $r = 1$ if there is a perfect positive correlation, and $r = -1$ if there is a perfect negative correlation. Values between -1 and 1 indicate an imperfect correlation. The p-value reported is an indicator of the probability that no correlation exists between the two parameters and that the coefficient results from mere chance. The correlation results are shown in table 3.4.6.2.2. The results indicate a strong correlation between the potency of PAH in the two mouse strains in the tumor initiation assays.

We also compared the potency of PAH in the initiation assay (CD-1 mice) to the potency in the complete carcinogenesis assay (C57BL mice), using the same process as above. We observed a good correlation between the two assays (table 3.4.6.1.3 and table 3.4.6.1.4). Our findings are consistent with what have been reported previously by Slaga and Fischer (1983). Some of the data used in our analysis are probably the same as those analyzed by Slaga and Fischer (these authors did not provide the source of their raw data or their methods of estimating PAH potency).

We conclude that the relative potency of PAH is very stable in different mouse skin tumor assays employing different mouse strains.

3.4.6.2 Relative Potency of PAH in Different Rodent Species and Tissues

This section investigates whether the relative potency of PAH is the same in different species and in different tissues. The following observations lead us to conclude that the relative potency of PAH is indeed similar in different species and in different tissues.

- The relative potency in the mouse tumor initiation assay correlates well with that in the rat lung assay for a number of PAH.
- The relative potency of 3-MC and B[a]P is very similar in mice, rats, and hamsters, regardless of the exposure route (oral, respiratory or dermal).

- The relative activity levels of the key activating and deactivating enzymes as well as the PAH metabolic profiles are similar in different tissues in animals.

The evidence is described in greater detail in the following subsections.

3.4.6.2.1 Relative Potency of PAH in Mouse Skin Model Compared to Rat Lung Model

We compared the relative potency of PAH in the mouse skin tumor initiation model and in the rat lung-implantation model (see table 3.4.6.2.1.1). Unfortunately, very few compounds have been tested in both assays. This partly explains why the correlation between the two tests is not statistically significant (see table 3.4.6.2.1.2), despite the apparent good correlation between the two sets of data shown in table 3.4.6.2.1.1. Nevertheless, assuming that the tissue type has no impact on the observed correlation (supported by the analysis in section 3.4.6.2.3), the results are consistent with the relative potency model for risk assessment proposed by Thorslund and Charnley (1988), and by Krewski *et al.* (1989).

3.4.6.2.2 3-MC to B[a]P Ratio

This section compares the potencies of B[a]P and 3-MC in three rodent species by three routes of exposure and evaluates if the relative potency is the same in all instances. The comparison can serve two purposes. Firstly, a positive finding indicates that the relative potency model for risk assessment holds for PAH regardless of species and routes of exposure. Secondly, a positive finding suggests that the technical problems resulting from using diverse data obtained from studies that were plagued with methodological differences and poor reporting are not significant enough to invalidate the results of the comparisons.

We compared the relative potency of B[a]P and 3-MC in mice, rats, and hamsters by oral, respiratory and topical exposure. The data are those obtained from section 3.4.4 and are provided in appendix A.

The assessment was conducted as follows.

- We selected from the literature studies with B[a]P and 3-MC that had similar experimental designs. For examples, for each comparison, the test compounds and treatment regimen were the same or similar. The only difference would be the route of exposure and/or the species exposed. The data for each combination of exposure route and species was selected from appendix A using the selection guidelines described in section 3.4.4.1.1.

Table 3.4.6.2.1.1 Relative sensitivity of CD-1 mice exposed topically and rats exposed by intralung application to PAH carcinogenicity.

Compound	CD-1 mice Skin Tumors [incidence/μg] (relative potency)	Rat Lung[a] Carcinomas [incidence/μg] (relative potency)	Rat/CD-1
Benzo[a]pyrene	5.798 (1)	0.225 (1)	0.039
Benzo[e]pyrene	0 (0)	0 (0)	NA
Chrysene	0.166 (0.029)	0.010 (0.044)	0.060
Dibenzo[a,h]anthracene	4.695 (0.810)	0.571 (2.540)	0.122
Phenanthrene	0.005 (0.0009)	0 (0)	0

[a] - Rat lung data from Deutsch-Wenzel *et al.* (1983, 1983b), Wenzel-Hartung *et al.* (1990), Grimmer *et al.* (1984, 1987).

Table 3.4.6.2.1.2 Correlation of PAH potency in CD-1 mice (topically exposed) and rats (intralung application). Mice were exposed by topical application (tumor initiation model) and rats by intralung application. Potency estimates were presented in table 3.4.6.2.1.

Parameter	Parameter Value
Pearson, r	0.795
p-value	0.1078
N	5

- We estimated the tumorigenic potency as incidence per mg of B[a]P or 3-MC administered.
- The relative potencies obtained with different routes of exposure for different animal species were compared.

Out of nine possible comparisons, we have been able to make only six (see table 3.4.6.2.2.1). Five of the six ratios were in very good agreement with each other, within the constraint of the methodological limitations. The one exception is respiratory exposure in mice, where B[a]P was less potent than 3-MC. It is not clear whether this difference is significant or merely due to differences in protocol between the two studies. The B[a]P study, for example, ran for 10 weeks, while the study with 3-MC lasted 21 to 24 weeks (see appendix A). The two studies also reported different types of tumors.

Table 3.4.6.2.2.1 Relative sensitivity of rodents to B[a]P and 3-MC carcinogenicity. Relative potency of the two compounds in the three species is expressed as the ratio of tumor incidence per mg for the two compounds.

Route	Relative Sensitivity - B[a]P/3-MC [(incid./$mg_{B[a]P}$) / (incid./mg_{3-MC})]			
	Mouse	Rat	Hamster	Geom. mean
Oral	3.2	5.9	5.6	4.7
Resp.	0.23	3.5	*	0.90
Skin	2.6		**	2.6
Geom. mean	1.2	4.5	5.6	

* - Rodents exposed to PAH via intrapulmonary implantation are more sensitive than when they are exposed by intratracheal instillation (unpublished). Since B[a]P was administered by intratracheal instillation and the only results available for 3-MC involved intrabronchial implantation, comparison cannot be made.

** - Hamsters do not develop tumors when exposed to B[a]P or 3-MC.

Overall, we conclude that the relative potency of B[a]P and 3-MC in different species administered by different routes is similar. The finding supports the notion that the relative potency approach holds for PAH across species and routes of administration. The results also provide evidence that the comparison between the studies is reliable enough to allow the estimation of the relative potency of PAH in different species and for different routes of exposure.

3.4.6.2.3 Pharmacokinetics

The metabolic activities of the epoxide hydrolase (EH), glutathione-S-transferase (GST) and P-450c (AHH or aryl hydrocarbon hydrolase) differ in different tissues. The activities tend to be highest in the liver, medium in the lungs and kidneys, and lowest in the intestines (see appendix D). On the other hand, the relative level of activities of the three enzymes appears to be similar within one order of magnitude in different tissues (see table 3.4.6.2.3.1 and data in appendix D). Furthermore, the profiles of metabolites formed in the liver and lungs of rats after B[a]P exposure via the respiratory tract are also similar (see table 3.4.6.2.3.2 and appendix D).

Table 3.4.6.2.3.1 Metabolic enzyme activities in various tissues relative to enzyme activities in rat liver.

Tissue	Enzyme Activity Relative to Liver (%)		
	AHH	EH	GST
Liver	100	100	100
Lung	3.4	5.3	7.7
Whole skin	1.4	1.8	Not available
Kidney	1.7	11	25
Intestine	0.34	2.0	Not available

AHH - aryl hydrocarbon hydrolase
EH - epoxide hydrolase
GST - glutathione-S-transferase

Table 3.4.6.2.3.2 Levels of B[a]P metabolites relative to levels of unmetabolized B[a]P in rat liver and lung 5 minutes post-treatment.

Metabolite	% Unmetabolized B[a]P		Lung/Liver
	Liver	Lung	
3-Hydroxy	1.3	1.7	1.3
9-Hydroxy	0.9	1.3	1.4
4,5-Diol	1.2	1.3	1.1
7,8-Diol	1.3	1.6	1.2
9,10-Diol	1.7	2.7	1.6
1,6-Dione	3.1	5.9	1.9
3,6-Dione	207	7.5	2.8
6,12-Dione	2.9	4.0	1.4
Conjugates	3.7	6.3	1.7

The above findings are consistent with the assumption that the relative potency of PAH is similar in different tissues, since the relative activity of the enzymes that activate

and inactivate PAH are similar and PAH metabolites are present in similar proportions in the tissues. The difference in the absolute enzyme activity levels in different tissues may account for the difference in the vulnerability of the various tissues to the tumorigenic effects of PAH.

3.4.6.3 Relative Potency of Mixtures

Section 3.4.6.3 compares the potencies of complex mixtures in one assay versus another assay, in one species versus another species, and by one route of exposure versus another route.

The relative potency model has been reported to hold not only for individual PAH (Thorslund and Charnley, 1988; Krewski *et al.* 1989), but also for mixtures (Albert *et al.* 1983). Albert *et al.* (1983) reported a good correlation between the potencies of mixtures (coke oven emissions, coal tar, and cigarette smoke) in humans and in animals when the potency was expressed in terms of the mass of the organic matter that constituted the mixture. His findings were supported by a few other authors (Lewtas, 1985, 1985b; Nesnow, 1989, 1990). Since we do not consider organic matter a good indicator for the potency of mixtures (see section 3.4.3.2.1), the issue of applicability of the relative potency approach to mixtures needs to be re-examined.

We selected for analysis mixtures for which potencies have been established in two different assays. For each mixture, we established the ratio of its potency in the first assay versus the second assay. If the relative potency assumption holds for mixtures, then the ratio of potencies in the same two assays would be similar for all mixtures. Tables 3.4.6.3.1 to 3.4.6.3.4 present the result of the comparison. Since the ratio is not only affected by the mixtures tested but also by the experimental protocol, the comparison is possible only with mixtures tested using similar methodologies. That explains why data from tables 3.4.6.3.1 to 3.4.6.3.4 cannot be combined.

Comparison was conducted in the following manner.

- humans versus mice, using the human data from Albert *et al.* (1983) (table 3.4.6.3.1)
- humans versus mice, using the estimates of human potency presented by Slooff *et al.* (1989) (table 3.4.6.3.2)
- mouse skin versus rat lung assays (table 3.4.6.3.3)
- mouse skin tumor initiation versus complete carcinogenicity assays (table 3.4.6.3.4)

The data summarized in the four tables show a good correlation between the potencies of the mixtures derived from different assay types. The correlation between mouse skin tumor initiation and complete carcinogenicity assays is less convincing than the other correlations. For example, Nissan diesel emissions were very potent in the tumor initiation assay, but inactive in

the complete carcinogenicity assay (see table 3.4.6.3.4). The apparent discrepancy is likely due to the low dose range the Nissan diesel emissions were tested in the complete carcinogenesis assay and is a consequence of a flaw in the experimental design (see section 3.3.2.1.1).

Table 3.4.6.3.1 Potencies of three mixtures in humans (based on data from Albert *et al.* (1983)) relative to a mouse skin assay. Human cancer potencies were reported per unit mass of organics, and have been converted to risk per unit mass of B[a]P content in the present report.

Mixture	Human Cancer Risk (/ng/m^3 B[a]P in mixture)	Mouse Tumor Incidence (/ng B[a]P in mixture)	Human Potency/ Mouse Potency
Coke oven emissions	5.6 E-4	0.25	2.2 E-3
Roofing tar	4.1 E-4	0.18	2.3 E-3
CSC	> 2.2 E-3	> 0.27	abt 8.2 E-3
Geom. Mean	8.0 E-4	0.23	3.5 E-3

Table 3.4.6.3.2 Potencies of two mixtures in humans relative to a mouse skin assay. Potencies of human mixtures were derived from table 3.4.6.3.1.

Mixture	Human Cancer Risk (/ng/m^3 B[a]P in mixture)	Mouse Tumor Incidence (/ng B[a]P in mixture)	Human Potency/ Mouse Potency
Coke oven emissions -topside	5.0 E-5	7.6 E-2	6.6 E-4
Smoky coal	6.6 E-5	7.8 E-2	8.5 E-4

Table 3.4.6.3.3 Relative potencies of mixtures in mouse skin and rat lung assays.

Mixture	Mouse Skin Complete Carc. (incidence/ng B[a]P)	Rat Lung (incidence/ng B[a]P)	Mouse Skin Potency/Rat Lung Potency
Automobile exhaust	3.2 E-4	1.0 E-2	3.2 E-2
Flue gas	6.9 E-4	1.7 E-2	4.1 E-2

Table 3.4.6.3.4 Relative potencies of mixtures in two mouse skin assays.

Mixture	Mouse Skin Complete Carcinogenesis (incidence/ng B[a]P)	Mouse Skin Tumor Initiation (incidence/ng B[a]P)	Complete Carc./Tumor Init. Potency
Smoky coal	2.5 E-4	7.8 E-2	3.2 E-3
Coke oven emissions -main	6.1 E-4	9.4 E-2	6.5 E-3
Roofing tar	6.9 E-5	1.1 E-2	6.3 E-3
Nissan diesel exhaust	*	2.4 E-2	*
Caterpillar diesel exhaust	0	0	NA
Oldsmobile diesel exhaust	0	0	NA

* See explanation in text.

Our analysis is limited because our data sets are small and comparison has to be made between data sets from different investigators. In some instances, different investigators might be studying mixtures from the same homologous source, but the composition of the mixtures could have been different. This has been the case, for example, in table 3.4.6.3.1 where coke oven emission samples used to study tumor initiation in mice may have been different from the emissions to which coke oven workers were exposed.

Based on the results, we conclude that the carcinogenicity of different mixtures correlate well for different species.

3.4.6.4 Relative Potency of Mixtures to B[a]P

This section examines whether the relative potencies of complex mixtures to B[a]P are the same regardless of experimental protocol, species, and route of administration.

We tested two assumptions. The first assumption states that the potency of a particular mixture type (e.g. diesel emissions, coal tars) relative to the potency of B[a]P tested under the same conditions is constant, regardless of the assay and species used and independent of the route of exposure. The second assumption is less constrained and states that for mixtures where unsubstituted PAH account for most of the overall tumorigenicity, the ratio of the potency of the mixture to that of B[a]P tested under the same conditions is the **same regardless of the mixture type**, assay, species and route of exposure.

In order to test the assumptions, we compared the potency of the mixture and the potency of B[a]P for a number of mixtures. The data were obtained from the published literature. Tables 3.4.6.4.1 and 3.4.6.4.2 summarize the results of the comparison.

Table 3.4.6.4.1 Relative potency of complex mixture to B[a]P in different assays.

Mixture type	Mixture	Assay	Mixture (incidence/ng B[a]P)	B[a]P (incidence/ng)	Ratio Mixture/B[a]P	Calculated from (ref.)
Diesels	Nissan diesel	tumor initiation	2.4 E-2	4.9 E-3	4.9	Nesnow, 1982
	VW diesel	tumor initiation	1.1 E-1	4.9 E-3	22	Nesnow, 1982
	Nissan diesel	complete carc.	*	3.5 E-5	*	Nesnow, 1982
	diesel	rat lung	5.8 E-3	2.0 E-4	29	Grimmer, 1987b
Gasoline engine	Mustang gasoline exhaust	tumor initiation	5.6 E-2	4.9 E-3	11	Nesnow, 1982
	gasoline exhaust	complete carc.	3.2 E-4	3.2 E-5	10	Grimmer, 1984
	gasoline exhaust	rat lung	1.0 E-2	2.3 E-4	44	Grimmer, 1984
Smoky coal	smoky coal emiss.	complete carc.	2.5 E-4**	1.9 E-4**	1.3	Mumford *et al.* 1990
	smoky coal emiss.	tumor initiation	7.8 E-2	1.6 E-3	49	Mumford *et al.* 1990
Coke oven	coke oven emissions (*Main*)	complete carc.	6.1 E-4	3.5 E-5	17	Nesnow, 1982
	coke oven emissions (*Main*)	tumor initiation	9.4 E-2	4.9 E-3	19	Nesnow, 1982
	coke oven emissions (*Topside*)	tumor initiation	7.6 E-2	4.9 E-3	16	Nesnow, 1982
Coal tar	roofing tar	complete carc.	6.9 E-5	3.5 E-5	2.0	Nesnow, 1982
	roofing tar	tumor initiation	1.1 E-2	4.9 E-3	2.2	Nesnow, 1982
Flue gas	flue gas	complete carc.	6.9 E-4	8.3 E-5	8.3	Grimmer, 1985
	flue gas	rat lung	1.7 E-2	2.0 E-4	85	Grimmer, 1987

* Nissan diesel emissions were tested in the complete carcinogenicity assay at a dose considered too low to induce a response. See section 3.4.6.3 for more detailed explanation.

** Slope calculated from two data points.

Table 3.4.6.4.2 Potency of cigarette smoke condensate relative to B[a]P.

Mixture	Assay	Mixture (incidence/ng B[a]P)	B[a]P (incidence/ng)	Ratio Mixture/B[a]P	Calculated from (ref.)
CSC	rat lung	> 5.0 E-1*	2.6 E-4	> 1900	Grimmer, 1988

* - <1 ng B[a]P/mg of organics assumed (Albert *et al.* 1983).

The potency of mixtures relative to B[a]P **within a mixture type** was relatively stable within one order of magnitude (see the sixth column, table 3.4.6.4.1). This finding provides evidence in support of our first assumption, that the potency of mixtures within a mixture type is independent of the assay, the species and the route of exposure.

Table 3.4.6.4.1 also shows that the potencies of mixtures correlate well with the potency of B[a]P in the same assay. The ratio of the potency of mixtures to that of B[a]P is stable within one and a half orders of magnitude, and is independent of the mixture type. Considering the variations in the experimental designs, species used, mixtures tested and the determination of the potency for both mixtures and B[a]P, we consider the findings a strong confirmation that the potencies of PAH-rich mixtures are comparable. The findings are, therefore, consistent with the second, more general assumption.

The only major outlier among PAH-containing complex mixtures is cigarette smoke condensate (CSC). The potency of cigarette smoke condensate relative to B[a]P differs from the relative potencies of other mixtures listed in table 3.4.6.4.1. The finding is within one's expectation, as CSC contains relatively low levels of both B[a]P and other PAH (see IARC, 1986), and much of the tumorigenicity of the mixture is due to compounds other than PAH, such as nitrosamines (see IARC, 1986). As a result, the mixture is relatively potent per mg of organics. Since the B[a]P levels are low, the potency of the mixture **per ng of B[a]P** will be high and thus the ratio of the potency of the mixture to the potency of B[a]P will also be high.

The mixtures listed in table 3.4.6.4.1 are generally considered PAH-rich and although the mixtures contain also other tumorigenic compounds, much of the tumorigenicity is attributed to PAH. Since the geometric mean of the ratio of the potency of PAH-rich mixtures to that of B[a]P is about 16 (table 3.4.6.4.1) and the ratio for most mixtures is well within an order of magnitude of 16, we assume that the ratio, 16, may apply to other PAH- rich mixtures. For CSC, the ratio is about 1900, therefore PAH is likely responsible for only approximately 1% of CSC tumorigenicity (16/1900*100).

Overall, we conclude that the relative potency of mixtures to B[a]P is relatively stable, independent of assays, species and route of exposure.

3.4.7 Route of Exposure and Potency of PAH

Although humans are usually exposed to PAH by the oral, dermal and inhalation routes, many risk assessments are limited to considering the inhalation route only. Other assessments assume that the potency and target site are the same regardless of the route of administration. The implicit assumption is that the exposures at a given site from different routes are additive and the risk is proportional to the total dose. This section examines if the route of exposure affects both the potency and the site of action for PAH. The findings that the effect is significant suggests the above approaches are inappropriate for evaluating health risk.

More sophisticated assessments take into consideration the route of exposure and determine the risk for each route of exposure independently (Schoeny *et al.* 1991; Thorslund and Farrar, 1990). The risk from oral exposure is usually determined from the mouse oral study by Neal and Rigdon (1967, see table 3.7.2.3.1). The result suggests that the PAH potencies by the oral and inhalation routes are similar (compare results in tables 3.7.2.1.1a, 3.7.2.1.1b and 3.7.2.3.1). We consider such similarities in potency unlikely, since human exposure to PAH by the oral route is considerably more substantial than by the inhalation route (see exposure assessment document), such that the risk induced by ingested PAH should overshadow the risk from all other exposure with the exception of the biggest occupational exposures. We find no indication of such happening in the literature.

The dermal route may be important for individuals exposed to high levels of PAH in the air, soil or sediments. This scenario is often considered in site-specific assessments, yet we find no risk assessments that attempt to estimate the dose-risk relationship for PAH for dermal exposure.

This section examines the difference in sensitivity of the different routes of exposure on the toxicity of PAH in animal species. If the difference between routes of exposure is consistent across species, then the same difference can be assumed for humans. This information can then be utilized to estimate human risk due to exposure by the oral and dermal routes from known inhalation health risk (see section 3.5.5).

3.4.7.1 Methods

In general, the procedure to establish the relative potency of PAH administered by different routes was similar to that described in section 3.4.6.2.2 for the comparison of the potencies of B[a]P and 3-MC. The data from appendix A were used for the calculation.

The assessment was conducted as follows.

- We selected from the literature studies with B[a]P and 3-MC of similar experimental design. For example, for each set of comparison, the test compounds and treatment regimen were the same or similar, and the only difference was the route of exposure. The data were selected from appendix A for each combination of exposure route and species using the selection guidelines described in section 3.4.4.1.1.
- We estimated the dose that would induce 50% incidence of tumors (ED_{50}).
- The ED_{50}s obtained for different routes of exposure were compared.

The dose predicted to induce 50% incidence of tumors was calculated by dividing the 50% incidence by the potency in incidence/mg previously determined for each species and route considered (equation 3.4.7.1.1).

$$estimated \quad ED_{50} = \frac{50\% \quad incidence}{observed \quad incidence/mg}$$

Equation 3.4.7.1.1

3.4.7.2 Results

3.4.7.2.1 Mice

The results are illustrated in figure 3.4.7.2.1.1 and table 3.4.7.2.1.1. In the mouse, 3-MC and B[a]P were much more potent when administered by the respiratory and topical routes than by the oral route. As a result, a much larger oral dose of B[a]P or 3-MC was required to induce 50% incidence of tumors. The dose-response curves for oral and topical applications of B[a]P are compared in figure 3.4.7.2.1.1. B[a]P is about two orders of magnitude more potent by topical, rather than oral administration.

In table 3.4.7.2.1.1, the orally administered B[a]P appears to be more potent than would be expected from the 3-MC data or from the full dose-response curves in figure 3.4.7.2.1.1. This discrepancy may stem from the exceptional sensitivity to oral B[a]P in the study of Wattenberg *et al.* (1979). The apparent discrepancy in relative potency between B[a]P and 3-MC may be a chance difference or it may stem from the difference in exper-

imental protocol between the 3-MC experiment (administered in drinking water) and B[a]P experiment (given by gavage). In a study where B[a]P was administered in the diet, B[a]P was roughly equipotent to 3-MC within one order of magnitude (see table 3.4.7.2.1.1).

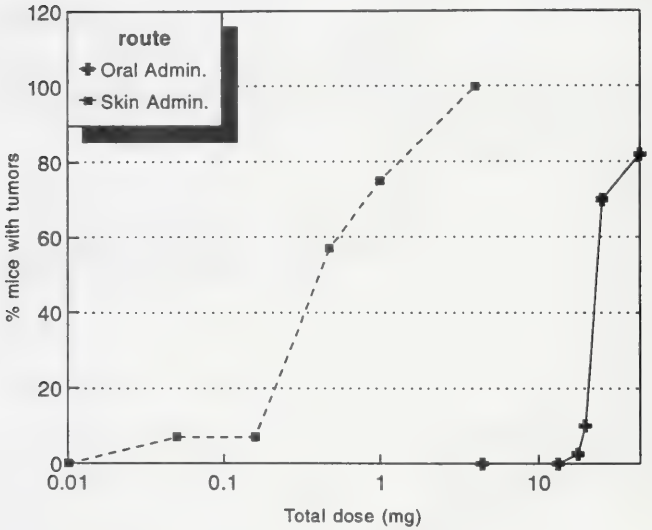

Figure 3.4.7.2.1.1 Relative sensitivity of mice to B[a]P by oral administration and by topical application in the complete carcinogenesis assay. From the data of Cavalieri *et al.* (1978, 1983).

Table 3.4.7.2.1.1 Effect of route of administration on potency of 3-MC (B[a]P) in mice.

Route	Estimated Dose at 50% Incidence (ED_{50}, mg)		Reference
	3-MC	B[a]P	
oral	57	3.8 (18*)	Lorenz and Stewart, 1948; Wattenberg *et al.* 1979; Neal & Rigdon, 1967*
respiratory	1.7	7.8	Nettesheim & Hammons, 1971; Furst *et al.* 1979
topical	1.1	0.42	Cavalieri *et al.* 1978, 1983

* The result within the parentheses was due to administration of B[a]P in the diet, and the result outside the parentheses was obtained by gavage. The diet value may be more closely comparable to the 3-MC value (3-MC administered in drinking water), and is therefore given greater weight.

3.4.7.2.2 Rats

The results for rats are summarized in table 3.4.7.2.2.1. The results indicate that 3-MC administered by the topical route is more than an order of magnitude more potent than when it was administered by the oral route. 3-MC or B[a]P administered by the respiratory route are two to three orders of magnitude more potent than 3-MC or B[a]P administered orally. In addition, there is a good agreement between the relative potencies obtained with 3-MC and B[a]P.

Table 3.4.7.2.2.1 Effect of route of administration on potency of 3-MC (B[a]P) in rats.

Route	Estimated Dose at 50% Incidence (ED_{50}, mg)		Reference
	3-MC	B[a]P	
oral	breast tumors only	64	Brune *et al.* 1989
respiratory	0.57	0.16	Hirano *et al.* 1974; Wenzel-Hartung, 1990
topical	31	no data	Zackheim, 1964

3.4.7.2.3 Hamsters

The results for hamsters are summarized in table 3.4.7.2.3.1. The results indicate that both 3-MC and B[a]P administered by the respiratory route is more potent than by the oral route. Topically administered 3-MC or B[a]P did not induce tumors.

Table 3.4.7.2.3.1 Effect of route of administration on potency of 3-MC (B[a]P) in hamsters.

Route	Estimated Dose at 50% Incidence (ED_{50}, mg)		Reference
	3-MC	B[a]P	
oral	1100	200	Homburger *et al.* 1972; Dontenwill & Mohr, 1962
respiratory	0.57	23	Hammond *et al.* 1987; Saffiotti *et al.* 1968
topical	no tumors	no tumors	Shubik *et al.* 1960

The results for 3-MC and B[a]P cannot be compared directly because B[a]P was administered by intratracheal instillation, and the only available data set for 3-MC involves *intrabronchial implantation (see section 3.4.7.2.3). It is impo*rtant to note that rodents exposed to PAH via intrapulmonary implant are more sensitive to the compounds than when they are exposed by intratracheal instillation (see section 3.3.2.1.3).

3.4.7.3 Pharmacokinetics

The pharmacokinetic data summarized in table 3.4.7.3.1 suggest that the relative amount of DNA adducts found in a given tissue is influenced by the route by which PAH is administered. This finding is consistent with our hypothesis that different tumorigenic potency of PAH at different sites is a function of the route of administration. The data and analysis are based on appendix D.

Table 3.4.7.3.1 Total DNA binding of labelled PAH metabolites in different tissues. All values presented as % of binding to lung tissues.

Route of Exposure	Liver	Skin	Lung	Forestomach
dermal	NA	650	100	NA
oral	57	NA	100	145
	117	45	100	NA
intratracheal	254	NA	100	NA

3.4.7.4 Discussion

The experimental results for 3-MC and B[a]P in all the animal species examined were pooled and summarized in table 3.4.7.4.1 and in figure 3.4.7.4.1.

We are unable to find individual studies that examined specifically the effect of the route of administration on the potency of PAH. Such studies would provide a relatively reliable basis on which route-to-route extrapolation can be conducted. In the absence of such studies, we have to rely on studies that have different experimental designs. Therefore, it is impossible to exclude the possibility that the reported difference is due to methodological differences rather than any genuine difference resulting from different routes of exposure.

As shown in figure 3.4.7.4.1 and table 3.4.7.4.2, the potencies of PAH administered by oral and respiratory routes differ substantially. The differences are consistent in all three rodents. Secondly, the results obtained with B[a]P generally agree with the data for 3-MC (see table 3.4.7.4.1). Finally, the pharmacokinetic data show that DNA binding in tissues varies as a function of the route of administration (see section 3.4.7.3). We therefore interpret the difference in potencies from different routes of exposure as authentic and not as a result of any methodological flaws.

It is apparent that administration by the oral route is less effective in inducing tumors than administration by the respiratory route (see table 3.4.7.4.2). For the three species we examined, the oral route is more than two orders of magnitude less effective than the respiratory route. Topical administration is less effective than administration to the respiratory system by about an order of magnitude for all three species. On the other hand, when compared with oral administration, topical application of 3-MC is clearly more potent in both rats and mice. Because of the significant difference between the relative sensitivity of routes of exposure to 3-MC, routes of exposure should always be considered throughout the risk assessment exercise.

Table 3.4.7.4.2 has several implications for risk assessment. It permits the estimation of PAH potency from one rodent species treated via a particular route to another rodent species treated by another exposure route. Table 3.4.7.4.2 also shows that the oral and the respiratory tract potencies of 3-MC are reasonably similar across the rodent species and the respiratory route is about 330 times more sensitive than the oral route. It may be prudent to give respiratory exposure 330 times more weight than oral exposure when human risk is being determined.

Table 3.4.7.4.1 Potency of 3-MC (B[a]P) in rodents associated with various routes of exposure. The most sensitive endpoints have been used for the comparison. Data from single administrations were excluded.

Route	Incidence/ng 3-MC (B[a]P)		
	Mouse	Rat	Hamster
Topical	46 (120)	1.6	*
Respiratory Tract	29 (6.6)	88 (310)	87 (2.2)
Oral	0.87 (2.8)	0.15 (0.79)	0.045 (0.25)

* - No effect at tested dose (0.5 mg)

Table 3.4.7.4.2 Relative potency of PAH in rodents associated with various exposure routes. The most sensitive endpoints have been used for the comparison. Potencies for various routes (in incidence/ng of 3-MC) were converted to potencies relative to the average potency in the respiratory tract.

Route	Potency Relative to Respiratory Tract			
	Mouse	Rat	Hamster	Geom. Mean
Topical	0.75	0.026	*	0.14**
Respiratory Tract	0.48	1.4	1.4	1
Oral	0.014	0.0029	0.00074	0.0031

* - No effect at tested dose (0.5 mg)

** - excludes hamster data

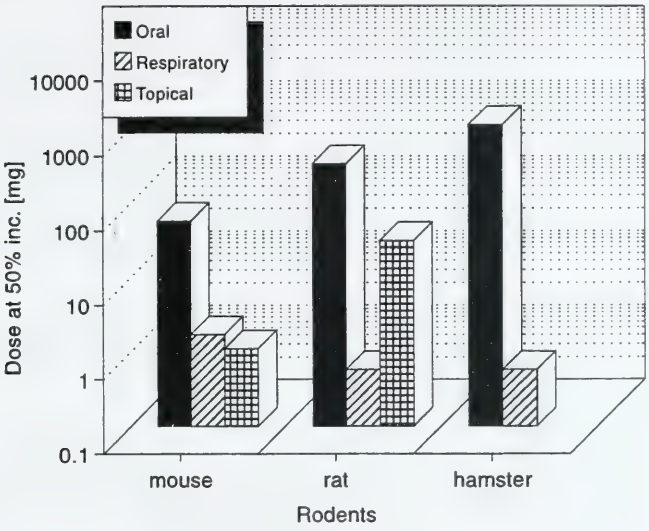

Figure 3.4.7.4.1 Dose of 3-methylcholanthrene (3-MC) required to induce tumors in 50 percent of test rodents via various routes of exposure.

PAH potency at most sensitive sites *versus selected sites*

Table 3.4.7.4.1 reports the relative responses at the most sensitive sites as described below.

- skin tumors with dermal exposure
- forestomach tumors with oral exposure
- respiratory tract tumors with respiratory tract exposure

We have selected the most sensitive tumor sites and defined them as the *main sites*. However, there are some exceptions.

- We did not find any reference to the appearance of forestomach tumors in rats after oral exposure to B[a]P or 3-MC. After oral exposure, breast tumors have been reported in female rats. The breast tumor data have been used to estimate the potency at the most sensitive site. The potency at the main site (stomach) for rats is zero.
- Mice have been reported by Furst *et al.* (1979) to develop adenomas much more readily than squamous cell tumors of the respiratory tract. However, other authors (Nettesheim and Hammons, 1971) reported squamous cell tumors at a lower dose range than Furst's study. We therefore consider squamous cell carcinomas as the most sensitive endpoint, although adenomas may possibly appear at an even lower dose range.
- Hamsters do not appear to develop skin papillomas or carcinomas as mice do, but rather develop dermal melanomas (Bernfeld and Homburger, 1983) after exposure to DMBA. Exposures to 3-MC or B[a]P do not elevate the rate of melanoma incidence.

We have defined the main tumor site for each of the three exposure routes. The main tumor sites are generally the most sensitive tumor sites with the exception of rats exposed to PAH by the oral route. In the case of rats, the most sensitive site is the breast of the female rats while the main site is the forestomach. However, we have not found reference to tumorigenicity at the forestomach on oral exposure. In addition, tumors generally develop at all the main sites on exposure by any route. The proportion of response at each main site differs for each route of exposure. The contribution of risk at a given site from multiple routes of exposure is the subject of the next section.

3.4.8 Selection of Tumor Sites

Section 3.4.8.1 discusses the effect of animal species, the route of administration and the dosing schedule on tumor site. The relevance of these findings to humans is discussed in section 3.4.8.2.

3.4.8.1 Tumor Sites in Rodents

In this section, we examine the location of tumor sites as a function of the species considered and the route of exposure. Since most data are available for mice, rats and hamsters, we selected these three species for consideration. Exposures via the respiratory tract, GI tract, and the skin are considered because of their relevance to human exposure to PAH.

The analysis is best conducted using data from studies that examine more than one tissue for possible PAH-induced increase in tumor incidence or multiplicity. However, since there are very few such studies, we have included all studies that reported any PAH-induced tumor. The data are summarized in table 3.4.8.1.1.

In general, tumors have been observed in the skin, GI tract (mostly forestomach), respiratory tract and female mammary tissues in more than one species when exposed to PAH. The main target site varies with the route of exposure.

Since the studies made no reference to tumors at any other site[16], the primary tumors after dermal exposure appear to be located in the skin and the mammary glands (table 3.4.8.1.1). Responses to topically applied PAH are also species-specific. Mice and rats tend to develop skin papillomas and squamous cell carcinomas. In contrast, hamsters develop skin melanomas after a single administration of DMBA (no promotion) and no tumors at all after treatment with B[a]P or 3-MC (Bernfeld and Homburger, 1983). Mice generally require promotion (Slaga, 1983).

Oral application of PAH seems to induce tumors mainly in the GI tract and mammary tissues in females. There are some species-specific differences. Rats do not seem to develop GI tract tumors as readily as mice and hamsters (Shay *et al.* 1949). In addition, mice develop respiratory tract tumors after oral exposure. This phenomenon has not been observed in the other rodent species.

Administration of PAH into the respiratory tract results primarily in respiratory tract tumors in all animal species. Thyssen *et al.* (1981) and Saffiotti *et al.* (1972) also observed tumors in the GI tract after exposing hamsters to PAH via the respiratory tract.

In summary, the results in table 3.4.8.1.1 suggest that the site of tumor development is strongly dependent on the route of administration. In addition, there is some indication that the responses are species-specific. Although table 3.4.8.1.1 presents sparse evidence for the effect of dosing schedule on the site of tumors, section 3.8.2 will discuss in detail the effect of dosing schedule on PAH potency at different tumor sites.

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16 We do not see this evidence as conclusive. It is possible that the investigators reported only the skin and mammary tumors because they were readily apparent without pathological investigation. Therefore, the investigators might have focussed their attention on skin tumors as a readily available measure of tumorigenic activity.

Table 3.4.8.1.1 Tumor sites as a result of PAH exposure: effect of species, route of administration and dosing schedule.

Route	Species	Dosing	Site of Tumor					Reference
			Skin	Mam	GI	RT	Leuk	
t.a.	mouse	initiation	4	4			1	Slaga *et al.* 1982; Cavalieri *et al.* 1978; Ranadive & Karande, 1963
t.a.	mouse	multiple	4					Slaga *et al.* 1982
t.a.	rat	multiple	4					Zackheim, 1964
t.a.	hamster	single	4					Bernfeld & Homburger, 1983
t.a.	hamster	initiation	*					Bernfeld & Homburger, 1983
t.a.	hamster	multiple	*					Bernfeld & Homburger, 1983
p.o.	mouse	multiple			4	3	2	Rigdon & Neal, 1966, 1969; Lorenz & Stewart, 1948
p.o.	mouse	single			4	3		Pierce, 1961; Stoner *et al.* 1984
p.o.	female rat	single		4				Hugging & Yang, 1962
p.o.	female rat	multiple		4	0			Shay *et al.* 1949
p.o.	hamster	single	4		3			Bernfeld & Homburger, 1983; Goerttler *et al.* 1980
p.o.	hamster	multiple		4	4			Homburger *et al.* 1972
i.t.	rat	multiple				4		Ishinishi *et al.* 1976; Blair, 1974; Maeda *et al.* 1986
i.l.	rat	slow rel.				4		Deutsch-Wenzel *et al.* 1983, 1983b
inhl	hamster	multiple			4	4		Thyssen *et al.* 1981
i.b.	hamster	slow rel.				4		Hammond *et al.* 1987
i.t.	hamster	multiple			2	4		Beems, 1984; Beems & van Beek, 1984; Yamamoto *et al.* 1985; Feron, 1972; Saffiotti *et al.* 1972

* - Tumors induced by DMBA, but not by B[a]P or 3-MC (see text).

t.a. = topical application
p.o. = oral administration
inhl = inhalation
i.t. = intratracheal administration
i.l. = intralung administration

i.b = intrabronchial administration
initiat = initiation- promotion assay
single = single dose administration
multiple = multiple doses administered
slow rel. = slow release

4 = main site
3 = important site
2 = unequivocal evidence for site
1 = equivocal evidence for site
0 = no tumors observed

3.4.8.2 Relevance to Humans

Rodent organs may differ significantly from human organs in both structure and function. Therefore, it is questionable whether one can assume that humans would develop tumor just because tumors have been observed in a particular tissue in rodents. In section 3.5.4.2, we have discussed some of the differences between the rodent and human respiratory systems. Human and rodent stomachs also differ in significant ways (Wester and Kroes, 1988; Clayson *et al.* 1990). The stomachs of rodents have evolved to digest plant material most efficiently. Rodents are continuous feeders, while humans typically eat meals at specific times of the day. As a result, the stomachs of healthy rodents are rarely empty, while human stomachs may be empty between meals. Unlike humans, rodents have a relatively large aglandular part of the stomach, called the forestomach. It is covered by squamous epithelium and does not contain acid secreting cells, which are found in the glandular area anterior to the pylorus. It is in the forestomach where most GI tract tumors are found in rodents.

On the other hand, for chemicals or groups of chemicals that have been recognized and widely accepted as human carcinogens, the concordance of tumor sites in humans with at least one of the two experimental animal species studied (rats or mice) is high, nearing perfection (Huff, 1993; Gold *et al.* 1992). The same assertion cannot be made for individual species. Prediction based on the target organ is roughly 47% from humans to rats and 37% from humans to mice (Gold *et al.* 1991). Furthermore, though mice and rats are two closely related species and are studied under similar experimental conditions, the overall prediction of positivity at some sites of one species is about 75% when the chemical produces a positive response in the other species. Site-specific prediction between rats and mice is less accurate, with approximately 50% chance of success (Gold *et al.* 1991; Huff *et al.* 1991). We interpret the findings in the following way for our assessment.

- We cannot assume that PAH that is found to induce tumors at a particular site in a single species will necessarily induce tumors at the same site in humans.
- Induction of tumors at the same site in two or three species by a PAH increases significantly the likelihood that the PAH would induce tumors at the same site in humans.

Since we have observed tumors in the skin, mammary tissues, respiratory and GI tract in two to three rodent species on exposure to PAH via various administration routes, we conclude that PAH may induce tumors in all four sites in humans in a similar manner as in the rodents.

3.4.9 Potency of PAH at Specific Tumor Site

3.4.9.1 Rationale

Exposure to PAH in different species by different routes of administration can lead to the development of tumors at different sites (see sections 3.4.8 and 3.4.7). In some instances, the same tumor site can be associated with more than one route of exposure (see section 3.4.9.2). This observation has practical consequence. For human populations, PAH from more than one medium (e.g. air, drinking water, soil) may interact to increase the risk of occurrence of tumor at a particular site. For example, PAH administered into either the respiratory or GI tract can increase the tumor incidence in the lungs (see sections 3.4.9.2.1 and 3.4.9.2.2). It is therefore important to determine the relative contributions of exposures through various routes to the tumor risk at a given site. To achieve this purpose, we made the following comparisons.

- Compare the tumor induction potency in the respiratory tract versus tumor induction potency in the forestomach. Exposure to PAH is by the oral route in both cases (section 3.4.9.2.1).
- Compare the tumor induction potency of PAH in the respiratory tract versus tumor induction potency in the forestomach (section 3.4.9.2.2). PAH is administered by the respiratory route in both cases.
- Compare the tumor induction potencies of PAH in the respiratory tract when administered by the oral route versus administration by the respiratory route (section 3.4.9.2.3).
- Compare the tumor induction potency of PAH in the forestomach when administered by the oral route versus administration by the respiratory route (section 3.4.9.2.3)

3.4.9.2 Methods and Results

PAH were known to induce tumors at more than one organ site. We compared the tumor induction potencies of B[a]P or 3-MC at different tumor sites for a given route of exposure in a given species. The data used were presented in appendix A.

The assessment was conducted as follows.

- First we selected a subset of data from appendix A for analysis. The goal of the selection process was to identify the most compatible data sets, where factors such as experimental animal species, test compound, and treatment regimen were the same or similar. The primary outstanding difference was the route of PAH administration.

- Next, we estimated the tumorigenic potency as incidence per mg of B[a]P or 3-MC where appropriate.
- Finally, the potencies at target tumor sites obtained with different routes of administration were compared.

Selection of data from appendix A for analysis followed the same guidelines as described in section 3.4.4.1.1.

3.4.9.2.1 Tumorigenic Potency of PAH in Respiratory Tract versus Forestomach on Oral Exposure

This section determines the tumorigenic potency of PAH in the forestomach relative to the respiratory tract as a result of oral exposure.

We selected from appendix A studies for which tumors were found in both the forestomach and the respiratory tract in animals which were administered PAH orally. These studies include studies by Lorenz and Stewart (1948), and Dontenwill and Mohr (1962). Although Pierce (1961) and Stoner *et al.* (1984) did not investigate tumor rates at both the forestomach and the respiratory tract, the two studies had compatible designs. We have therefore combined the Pierce and Stoner data for analysis.

Table 3.4.9.2.1.1 Potency (expressed as incidence/mg of PAH) in forestomach relative to potency of the same compound in lungs after oral administration.

Species	Route	B[a]P or 3-MC	Forestomach/Lung Relative Potency	Reference
mouse	oral, drinking water	3-MC	2.8	Lorenz & Stewart, 1948
mouse	single dose, oral	B[a]P	16	Pierce, 1961; Stoner *et al.* 1984
hamster	p.o.	B[a]P	46*	Dontenwill & Mohr, 1962
Geom. mean			13	

* No effect on trachea, although the incidence of tumors in the forestomach was 46% in the same animal species. We assumed a 1% incidence rate in the trachea.

The results are summarized in table 3.4.9.2.1.1. The forestomach is more sensitive to the action of PAH than both the lungs or the trachea. For the hamster, within the dose range tested, the doses that led to high tumor incidence rates (46%) in the forestomach did not induce tumors in the trachea. In the mouse, the geometric mean ratio of tumor potency in the forestomach to the potency in lungs for the two data sets was 13. The finding suggests that PAH is about thirteen times more effective in inducing tumors in the forestomach than in the lungs. For the purpose of the present assessment, we assume that orally administered PAH are about 13 times more potent in inducing tumors in the forestomach than in the respiratory tract.

3.4.9.2.2 Tumorigenic Potency of PAH in Respiratory Tract versus Forestomach on Exposure via Respiratory Route

This section determines the tumorigenic potency of PAH in the respiratory tract relative to the forestomach after exposure via the respiratory system.

We selected for the analysis studies from appendix A. In these studies, PAH induced tumors in both the forestomach and the respiratory tract after the animals were administered PAH via the respiratory tract. The two studies were those of Saffiotti *et al.* (1972) and Thyssen *et al.* (1981). The data were grouped according to the two different modes of PAH administration, intratracheal implantation and inhalation.

Table 3.4.9.2.2.1 Tumor potency (expressed as incidence/mg PAH) in lungs relative to potency in forestomach after PAH is administered into the respiratory tract.

Species	Route	B[a]P or 3-MC	Lung/Forestomach Relative Potency*	Reference
Hamster	intratracheal	B[a]P	4.3	Saffiotti *et al.* 1972
Hamster	inhalation	B[a]P	8.5**	Thyssen *et al.* 1981
Geom. mean			6.1	

* - (% incidence/ng in respiratory tract)/ (% incidence/ng in forestomach)
** - tumors in upper respiratory tract (no effect observed in lungs).

Table 3.4.9.2.2.1 summarizes the results of the analysis. The respiratory tract was more sensitive than the forestomach in both studies. The geometric mean of the ratio for the two mouse data sets was 6.1, suggesting that PAH was about six times more potent in inducing

tumors in the respiratory tract than in the forestomach. For the purpose of the present assessment, we assume that PAH are about six times more potent in inducing tumors in the respiratory tract than in the forestomach when exposure occurs via the respiratory tract.

3.4.9.2.3 Route of Exposure and Site-Specific Potency

Humans are often exposed to PAH via the oral and respiratory routes. Although we have provided evidence that the main target sites for the two routes of exposure are different (see section 3.4.7), both oral and respiratory exposure to PAH can lead to development of tumors in both the respiratory tract and the forestomach in exposed animals (see sections 3.4.9.2.1 and 3.4.9.2.2). As a result, both routes of exposure need to be considered during assessment of risk at a particular organ site. The relative sensitivity of the main sites[17] to the tumor inducing property of PAH by various routes of exposure (compared to exposure via the respiratory tract) was estimated in table 3.4.7.4.2. We further elaborated table 3.4.7.4.2 to include the secondary sites[18](see tables 3.4.9.2.1.1 and 3.4.9.2.2.1). The summary results, including both the main and secondary sites, are presented in table 3.4.9.2.3.1.

From tables 3.4.7.4.2, 3.4.9.2.1.1 and 3.4.9.2.2.1, we derived the following relationship between potencies.

$$lng_{res} = 1$$

$$stm_{orl}/lng_{res} = 0.0031 \qquad \text{equation 3.4.9.2.3.1}$$

$$stm_{orl}/lng_{orl} = 13 \qquad \text{equation 3.4.9.2.3.2}$$

$$lng_{res}/stm_{res} = 6.1 \qquad \text{equation 3.4.9.2.3.3}$$

where:

stm = the relative potency of PAH in the stomach compared to the potency in the lungs on exposure by respiration

lng = the relative potency of PAH in the lungs compared to the potency in the lungs on exposure by respiration

---

17 The main site is the site where tumors appear at the lowest administered dose of PAH, and is dependent of the route of exposure.

18 The secondary sites are the sites where tumors appear after exposure to higher levels of PAH than those needed for the main sites.

res = the respiratory route

orl = the oral route

For example, using equations 3.4.9.2.3.1 and 3.4.9.2.3.2, we estimated the potency of PAH at the lungs by the oral route relative to the lungs by respiratory route to be 0.0031/13 or 2.4 E-4. And using equation 3.4.9.2.3.3, the relative potency of PAH in the stomach by respiratory exposure was found to be 1/6.1 or 0.16 when compared to the potency in the lungs by the same route of exposure.

The results in table 3.4.9.2.3.1 will be used to extrapolate human potency from one route of exposure to another and from one tumor site to another. (see sections 3.5 and 3.6).

The results indicate that while respiratory exposure is most effective in inducing lung tumors, it is less effective in inducing forestomach tumors. Topical exposure, on the other hand, is as effective in inducing skin tumors as respiratory exposure in producing stomach tumors. Oral administration is least able to induce tumor for PAH. It is much less effective in inducing both forestomach and respiratory tract tumors than exposure via the respiratory tract. In effect, oral exposure contributes very little to the overall risk of both forestomach and lung tumors per unit PAH exposure.

Table 3.4.9.2.3.1 Relative potencies of PAH at different organ sites as a function of route of administration. Potency in respiratory tract after respiratory exposure is set to 1.

Route	Forestomach	Respiratory Tract	Skin
Topical	-	-	0.14
Respiratory	1.6 E-1	1	-
Oral	3.0 E-3	2.4 E-4	-

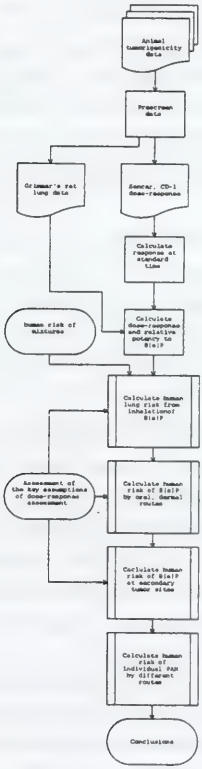
3.5 Dose-Response Assessment Based on Individual PAH Model (IPM)

3.5.1 Navigation Aid

This is the beginning of part c which consists of sections 3.5 to 3.7 and covers the dose-response assessment process for PAH. Figures 3.5.1.1 and 3.6.1.1 are provided to help the reader relate part

c to the other parts of the dose-response assessment document. Part c describes the two models which are considered and used for estimating the contribution of PAH to the risk of mixtures. Section 3.5 describes the IPM model and section 3.6 discusses the WMM model.

Figure 3.5.1.1 Dose-response assessment using IPM - a process flow diagram.


Animal tumorigenicity data
Prescreen data
Grimmer's rat lung data
Somcar, CD-1 dose-response
Calculate response at standard time
Human risk of mixtures
Calculate dose-response and relative potency to B(a)P
Calculate human lung risk from inhalation of B(a)P
Assessment of the key assumptions of dose-response assessment
Calculate human risk of B(a)P by oral, dermal routes
Calculate human risk of B(a)P at secondary tumor sites
Calculate human risk of individual PAH by different routes
Conclusions

3.5.2 Introduction

In the Individual PAH Model (IPM), the tumorigenicity of each individual PAH is expressed in terms of its relative potency to B[a]P so as to derive *B[a]P equivalents*. A compound twice as potent as B[a]P, for example, would have a relative potency of 2 B[a]P equivalents. In order to estimate the activity of a particular mixture or its PAH fraction, the quantity of each individual PAH in the mixture has to be determined, and translated into B[a]P equivalents. The B[a]P equivalents of all PAH present in the mixture are then summed up. Such a mixture is assumed to have the same potency as B[a]P of the same concentration.

This approach is also known as the *comparative potency approach* (Thorslund and Charnley, 1988) and the B[a]P equivalents are known as *toxic equivalency factors* (TEFs; see Nisbet and LaGoy, 1992). A number of authors have used the comparative potency approach or a modified version of the approach (Rugen *et al.* 1989; Krewski *et al.* 1989; Schoeny *et al.* 1991) in their risk assessments.

The dose-response assessment using the IPM model consists of several steps. The steps are outlined below.

- **Estimate the lifetime human lung cancer risk as a function of B[a]P airborne concentration.**

  The first step involves the estimation of B[a]P cancer potency in humans based on the lung cancer potency estimated for a PAH-rich complex mixture in exposed humans. The extrapolation is conducted using the relative potency approach (see section 3.4.6). The process is described in section 3.5.3.

- **Estimate the lung cancer risk as a function of B[a]P delivered to the lungs.**

  We convert the risk estimate obtained from the first step to an expression of intake into the lungs. In other words, instead of expressing the potency of B[a]P in terms of airborne levels, we express the potency in terms of the mass of B[a]P delivered to the lungs. The process is described in section 3.5.4.

- **Estimate the cancer potency of B[a]P for different exposure routes in humans**

  We estimate the potencies of B[a]P by oral and dermal routes by extrapolating from the inhalation route using the relative potency approach. The extrapolation is based on the relative potency of B[a]P and 3-MC of different exposure routes in rodents (relative to respiratory exposure). For further discussion of the data, see sections 3.4.7 and 3.4.9. The extrapolation process is described in section 3.5.5.

- **Estimate the potency of PAH relative to B[a]P in rodents**

  Using mouse skin and rat lung data (see sections 3.3.2.2.3 and 3.3.2.2.4), we develop the dose-response curves for each PAH from each assay. Next we estimate the potency for each compound in each assay relative to B[a]P. After we have verified that there is a good correlation between the relative potencies derived from the four assays, we combine the results to create a single set of estimates of potencies of PAH relative to B[a]P in rodents. The estimation is described in section 3.5.6.

- **Estimate the cancer potency of PAH for different exposure routes in humans**

  The last step involves the estimation of the cancer potency of PAH at different tumor sites for different exposure routes in exposed humans using the relative potency approach. Details are described in section 3.5.7.

3.5.3 Human Cancer Risk as a Function of Airborne B[a]P Concentration

This section estimates the human inhalation risk from exposure to B[a]P in air using the relative potency approach discussed and validated in section 3.4.6. In essence, we assume that the potency of B[a]P in humans relative to its potency in mouse tumor initiation assay is the same as the potency of a PAH-rich complex mixture in humans relative to its potency in the same mouse assay. The relationship can be summarized by equation 3.5.3.1.

$$C = \frac{human\ \ risk\ \ (PAH\ \ 1)}{exper.\ \ potency\ \ (PAH\ \ 1)} = \frac{human\ \ risk\ \ (PAH\ \ 2)}{exper.\ \ potency\ \ (PAH\ \ 2)} = \frac{human\ \ risk\ \ (mixt.1)}{exper.\ \ potency\ \ (mixt.1)} \qquad \text{Equation 3.5.3.1}$$

In order to use the relative potency approach to estimate the human risk due to B[a]P, we need the potency of a PAH-rich complex mixture and of B[a]P in the tumor initiation assay as well as the risk associated with the same mixture in humans. The animal data were obtained from Mumford *et al.* (1990). The human data were selected from the studies listed in table 3.3.1.1. The selection criteria are discussed in the following paragraphs.

There are a number of data sets that could have been used to estimate the potency of PAH-rich mixtures in humans (see section 3.3.1 and Slooff *et al.* 1989 for review of the data and assessments). Section 3.4.3.2.2.2 provides evidence that the potency of the PAH fraction of different complex mixture is approximately the same when the potency is expressed in terms of the B[a]P content of the mixture. The evidence is consistent with the results of the various human risk assessment studies (summarized in table 3.3.1.1) which arrived at a similar risk estimate for different mixtures. We have chosen the assessment of coke oven workers (USEPA, 1984) for the following reasons.

- Coke oven emissions have a very high PAH content (1.7 mg/g of organics, Albert *et al.* 1983). The high PAH content, together with the large occupational exposures, make PAH likely to be the major cause of lung tumors in the study subjects.
- The epidemiological studies on coke oven workers and the US EPA (1984) risk assessment based on the coke oven workers data have been generally accepted by the scientific community after extensive peer review.
- The results are consistent with findings in other epidemiological studies and in experimental rodent studies.

The US EPA risk assessment reported the estimated human lung cancer risk due to coke oven emissions in terms of risk per unit airborne concentration of benzene soluble fraction of the mixture. We converted the US EPA risk estimates into expressions of cancer risk in terms of B[a]P content. The results of unit conversion of the US EPA estimates are summarized in table 3.5.3.1. Based on our calculation, the human lifetime lung cancer risk from exposure to coke oven emissions is 2.3 E-5 per ng B[a]PS/m^3 [19](MLE). The upper confidence limit (UCL) on this estimate is 3.6 E-4 per ng B[a]PS/m^3.

Table 3.5.3.1 Estimated human lifetime lung cancer risk from exposure to coke oven emissions.

Mixture	B[a]P Content (ng/mg)	Risk (/mg organics/m^3)	Risk (/ng B[a]PS/m^3)	Risk (/ng B[a]PS/day)
Coke oven, UCL	1.7 E+03*	6.2 E-01[a]	3.6 E-04	2.2 E-04
Coke oven, MLE	1.7 E+03*	3.9 E-02**	2.3 E-05	1.4 E-05

* - Topside, rounded from Albert *et al.* 1983.

** - Determined the same way as reported for UCL by taking the geometric mean of the four MLE values reported in USEPA (1984). The four MLE values were obtained using different assumptions on the lag time.

[a] - USEPA (1984).

19 Note that B[a]PS is a surrogate for all PAH that have four to seven benzene rings in the mixture. Its potency is thus not that of B[a]P, but of most PAH which are present in coke oven emissions and which contribute to lung cancer. The potency of B[a]P is by definition less than the potency of B[a]PS, because B[a]P represents the potency of only one compound, while B[a]PS represents the potency of the mixture as a whole.

We calculated the potency of B[a]P from the potency of coke oven emissions in humans (table 3.5.3.1) using equation 3.5.3.2. The equation was derived by rearranging the terms in equation 3.5.3.1. The tumor initiation data for both coke oven emissions and B[a]P used in the calculation were derived from Nesnow *et al.* (1982) and are shown in table 3.5.3.2.

$$B[a]P_{Humans,\ Inhalation} = Mixture_{Humans,Inhalation}/Mixture_{Mice,Topical} * B[a]P_{Mice,Topical} \qquad \text{Equation 3.5.3.2}$$

The MLE human cancer risk for B[a]P can be calculated from the MLE human cancer risk from coke oven emissions in table 3.5.3.1 as illustrated by equation 3.5.3.3.

$$2.3\text{ E-}5/7.6\text{ E-}2 * 4.9\text{ E-}3 = 1.5\text{ E-}6 \qquad \text{Equation 3.5.3.3}$$

Table 3.5.3.2 Estimated human lifetime lung cancer risk from exposure to B[a]P.

B[a]P Source	Potency of Coke Oven Em. in SEN-CAR Mice (incid./ng B[a]PS)	Potency of B[a]P in SENCAR Mice (Incid./ng B[a]P)	Est. Lung Cancer Risk from B[a]P in Humans (/ng B[a]P/m^3)	Est. Lung Cancer Risk from B[a]P in Humans (/ng B[a]P/day)
Based on coke oven UCL	7.6 E-02*	4.9 E-03	2.4 E-05	1.4 E-05
Based on coke oven MLE	7.6 E-02*	4.9 E-03	1.5 E-06	8.8 E-07

* - topside

[a] - current SCD

Conversion factor to convert airborne levels of PAH to lung intake:

lifetime inhalation intake of B[a]P at 1 ng/m^3 (given in mg)	4.3 E-02[a]
daily inhalation intake of B[a]P at 1 ng/m^3 (given in ng)	1.7 E+00

In conclusion, the human lifetime lung cancer risk due to inhaled B[a]P is estimated to be 1.5 E-6 per ng B[a]P/m^3. The UCL on the estimate is 2.4 E-5 per ng B[a]P/m^3.

3.5.4 Human Cancer Risk as a Function of B[a]P Delivered to Lungs

The human lung cancer potency due to inhalation of B[a]P calculated in section 3.5.3 is expressed in terms of the airborne particulate-bound B[a]P concentration. In order to estimate the cancer potency of B[a]P in humans due to another route of exposure using the relative potency

approach, we need two additional pieces of information. The information we need are the lung tumor potency of B[a]P in an animal species as well as the route-specific tumor potency of B[a]P in the animal. Laboratory studies generally administer free PAH (i.e. not bound to particulates) to the animals through the lungs, the GI tract or the skin. Since the B[a]P administered in the animal lung assay was particulate-free, the potency derived from the experimental data is that of free B[a]P. Therefore, it is necessary to express the cancer potency in humans due to inhalation of B[a]P in air as a function of the level of particulate-free B[a]P deposited in lungs.

Section 3.5.4 describes how the human lung cancer potency of B[a]P due to inhalation of contaminated air was converted from an expression of airborne particulate-bound B[a]P concentration to an expression of bioavailable B[a]P levels in the lungs. The conversion process consisted of several steps.

- Calculate the total intake (amount inhaled) of B[a]P for a typical individual who is exposed daily to particulate-bound B[a]P in the air at a concentration of 1 ng/m^3 over his/her lifetime (section 3.5.4.1).
- Calculate the total amount of inhaled B[a]P which is available for absorption or transport into the lung tissues or tissue fluids for a typical individual who is exposed daily to particulate-bound B[a]P in the air at a concentration of 1 ng/m^3 over his/her lifetime (section 3.5.4.2). (Note section 3.5.4.2 also estimates the fraction of inhaled B[a]P that is transported to the GI system.)
- Calculate the lung cancer potency of bioavailable B[a]P in humans from inhalation (section 3.5.4.3).

3.5.4.1 Intake of B[a]P

The human health risk is expressed as lifetime risk/ng B[a]P/m^3. The mass of B[a]P inhaled over a lifetime can be derived from the equation 3.5.4.1.1 (based on USEPA, 1989).

IN = CA (ng/m^3) x IR (m^3/hr) x ET (hrs/d) x EF (d/yr) x ED (yr/life) Equation 3.5.4.1.1

where:

IN = ng of B[a]P inhaled over lifetime
CA = B[a]P concentration in the air
IR = inhalation rate (assume 17 m^3/24 hours or 0.71 m^3/hr - see exposure assessment document)
ET = exposure time in a day (24 hours)
EF = exposure frequency. Number of days in a year that exposure occurs (365 days assumed).
ED = 70 years (USEPA, 1987)

Example 3.5.4.1.1:
If CA = 1 ng/m^3,
then IN = 1 x 0.71 x 24 x 365 x 70 = 4.3 x 10^5 (ng)

Therefore, a hypothetical person breathing an average of 1 ng/m^3 B[a]P over a 70 year lifetime is expected to inhale about 0.43 mg of B[a]P. (Note: This is a conversion factor. The quantity inhaled is assumed to rise proportionally with the levels in the air. This calculation does not imply that Ontarians are on average exposed to 1 ng/m3.)

3.5.4.2 Bioavailable Fraction of B[a]P

Not all inhaled particulate-bound B[a]P (as with other PAH) are deposited in the respiratory tract, as some are exhaled again. Furthermore, some of the deposited PAH are not absorbed into the respiratory tract tissues, but are transported into the GI tract via mucociliary transport. We estimated the amount of B[a]P (and other PAH) available for uptake in the respiratory tract and the GI tract based on the modified ICRP 1979 model. The details of the calculation and the references used are contained in the inhalation exposure assessment document. Only the main assumptions and conclusions will be outlined in the present document.

The amount and the site of deposition of airborne particulates depend on the particle size and the type of breathing. We estimated the amount of deposition for particle size 2.5 μm, the median airborne particle size which are well within the range associated with PAH (see inhalation exposure assessment).

The type of breathing affects the site of particle deposition. Breathing through the mouth favours deposition in the lungs, while nasal breathing favours deposition in the upper respiratory tract. Most individuals breath through the mouth when their levels of physical activity are elevated, and through the nose at rest or during low level physical activities (see inhalation exposure assessment). We conclude that most Ontarians likely rely on nasal breathing most of the time and therefore assume nasal breathing as the default.

Clearance from the nasal and tracheobronchial parts of the respiratory tract is fast and we assume that all particulate-bound PAH deposited in these regions are transported into the GI tract (see inhalation exposure assessment). We also assume that 45% of the material deposited in the lungs will be absorbed in the lungs. The rest will be transported by macrophage transport to the upper parts of the respiratory tract and eventually to the GI tract or body fluids.

In theory, the PAH transported from lungs to the GI tract where they are then absorbed could contribute to the lung tumor risk, but it is not very likely to occur. In table 3.4.9.2.3.1, we estimated that PAH were about 4200 times more potent in inducing respiratory tumors when the

exposure was by respiratory route rather than by oral route. We therefore assume that the risk of lung cancer from inhaled PAH is attributable largely to those PAH absorbed from lungs and that the PAH transported to the GI tract contribute negligible lung cancer risk.

The amount of PAH bioavailable to the lungs from inhalation exposure can be derived from equation 3.5.4.2.1.

$$P_b = F_b \times F_r \times F_d \times F_a \times IN \qquad \text{Equation 3.5.4.2.1}$$

where:

P_b = PAH bioavailable to the lungs (ng)
F_b = Fraction of PAH bound to the particulate in the ambient air
F_r = Fraction of PAH-containing particulate that is respirable
F_d = Fraction of respirable particulate which is deposited in the lungs
F_a = Fraction of total deposited PAH that is not cleared from the lungs
IN = B[a]P inhaled over a lifetime

The individual parameters were evaluated as described below.

F_r

Pott (1983) has reviewed the literature and concluded that most PAH were associated with particles of 7 µm and less (see example from Bjørseth's data in table 3.5.4.2.1). Since particle size of 10 µm and less are readily respirable in humans (Schlesinger, 1989), we assume that *Fr is about 1* (See inhalation exposure assessment document for further discussion).

Table 3.5.4.2.1 Particle size (diameter) and B[a]P content. Adapted from Bjørseth *et al.* (1978).

Particle Size (µm)	B[a]P Content (% of total)
> 15	0.11
7-15	0.64
3-7	28
0.9-3	64
< 0.9	7.5

F_b

Under normal atmospheric conditions, most larger molecular PAH including B[a]P are largely bound to the particulate (Dann, 1992). Therefore, we assume that *Fb approaches 1* (see inhalation exposure assessment document for further discussion).

F_d and F_a

The product of the two parameters were estimated based on the ICRP 1979 model (see exposure assessment document). We modified the deposition rates from the model estimates to account for the difference between the distribution of particulates in the respiratory tract during high and low levels of physical activity (see table 3.5.4.2.2)

Table 3.5.4.2.2 Deposition rate of respirable particulates estimated from the data summarized by Schlesinger (1988). Independent estimation was conducted for nasal and oral breathing, based on particle size of 2.5 μm in diameter. Estimates were compared to values in the ICRP 1979 report.

Type of Breathing	Deposition (% inhaled)			
	Total	N-P*	T-B**	P***
Nasal	90	63	5	22
Oral	80	3	18	59
ICRP, 1979	63	30	8	25

* Nasal passage
** Tracheobronchial tree
*** Pulmonary parenchyma

During low activity, nasal breathing predominates in humans and more particulates are deposited onto the upper parts of the respiratory tract. In contrast, oral breathing leads to higher deposition of particulates in the lungs.

From the respiratory tract, most of the particulates are rapidly transported to the GI tract via mucociliary transport. We therefore assume that most of the PAH would not have time to desorb in the upper respiratory tract and those that do would end up in the GI tract. Nonetheless, some PAH, though a small proportion, are expected to desorb from the particulate and be available for uptake through the lung tissues. We estimated that about 45% of the PAH deposited into the lungs were taken up into the lungs or the lymphatic tissues.

We estimated the default value for $F_d \times F_a$ at 0.099. This represents all of the B[a]P that is deposited into the lungs during nasal breathing. For oral breathing, we assumed $F_d \times F_a$ to be 0.27. The latter value is more applicable to an occupational setting where workers are involved in physically demanding manual labour.

Taking into account the values of F_b, F_r, and $F_d \times F_a$, we can estimate the percentage of inhaled PAH particulates that are available for absorption in the GI tract and the lungs. Table 3.5.4.2.3 presents the estimation based on both the original ICRP (1979) model and a modified ICRP model. The modified model assumes that all particle-bound PAH are transported from the tracheobronchial tree and nasal part of the respiratory tract into the GI tract. It bases its assumption regarding the deposition rate of inhaled particles in the respiratory tract on more recent data than the original ICRP model (see exposure assessment document for details). Therefore, percentage absorptions calculated using the modified model will be used in the present assessment.

Table 3.5.4.2.3 Percentage of inhaled PAH predicted to be present in the GI tract and in the lungs using the original ICRP (1979) model and a modified ICRP model.

Compartment	% of Inhaled PAH that is Bioavailable		
	ICRP	Nasal Breathing	Oral Breathing
Body Fluids	12	NA	NA
GI*	36	81	62
Lung	11	9.9	27

* - particulate bound and not necessarily completely bioavailable.

In section 3.5.4.1, we estimated that a person breathing 1 ng of B[a]P per m^3 in air over a 70 year lifetime inhaled 0.43 mg of B[a]P. If a person is engaged in heavy labour over a lifetime, 0.27 x 0.43 mg or 0.12 mg of B[a]P would be bioavailable to the lungs for an airborne B[a]P concentration of 1 ng/m^3. If a person is engaged in light activity, we expect that about 0.099 x 0.43 mg or 0.043 mg will be bioavailable over a lifetime.

3.5.4.3 Risk from Bioavailable B[a]P in Lungs

In section 3.5.4.2, we estimated that over a lifetime, 0.043 mg of B[a]P was bioavailable in the lungs when humans inhaled air at a B[a]P concentration of 1 ng/m^3, assuming a low level of physical activity throughout most of the entire lifetime. In section 3.5.3, we estimated that the lifetime risk per ng B[a]P/m^3 was 1.5 E-6. Therefore, the lung cancer risk due to 1 ng of

bioavailable inhaled B[a]P in lungs over a lifetime would be 1.5 E-6/4.3 E4 or 3.5 E-11. This corresponds to a lifetime lung cancer risk of 8.8 E-7 risk/ng/day from inhalation of B[a]P in humans (summarized in table 3.5.5.1).

3.5.5 Risk from Exposure to B[a]P by Dermal and Oral Routes

This section estimates the potency of B[a]P at both the main and secondary sites (see section 3.4.9.2.3 for definition) for all three routes of exposure in humans. The process is described below.

Equation 3.5.5.1 was used to calculate the potency of B[a]P at a target site due to a particular route of exposure. The risk of lung cancer from respiratory exposure to B[a]P in humans over a lifetime (see sections 3.5.3 and 3.5.4) was multiplied by the relative potencies established for B[a]P from experimental animals for the exposure route of interest (summarized in table 3.4.9.2.3.1). The results are summarized in table 3.5.5.1.

$$B[a]P_{Humans,route} = B[a]P_{Humans,Inhalation} / \text{Conversion Factor}_{Route,species} \qquad \text{Equation 3.5.5.1}$$

Table 3.5.5.1 Human risk estimates (MLEs) expressed as risk/ng B[a]P/day at different sites and by different routes of exposure. B[a]P in this context is the fraction of the compound that is particulate-free.

Route	Site of Tumor		
	Stomach	Lung	Skin
Topical			1.2 E-7
Respiratory	1.4 E-7 or 2.4 E-7 (/ng/m^3)	8.8 E-7 or 1.5 E-6(/ng/m^3)	
Oral	2.6 E-9	2.1 E-10	

Rodents are not miniature humans. While forestomach tumors have been observed in rodents, no human equivalent of the forestomach exists. Nevertheless, we have used forestomach data as a predictor of tumors in the human GI system. Similarly, the rodent respiratory system differs from the human system. Experimental rodents were exposed to PAH at the level of the trachea and bronchi instead of the nose during inhalation. Tumors were likewise seen in different parts of the respiratory tract depending on the method used and other factors. We assume that the various experiments are predictive of human lung cancer. Therefore, whenever we are not specifically describing particular

experimental rodent data, but rather referring to extrapolation to a human condition, we use the terminologies *lung tumors, stomach tumors* and *inhalation exposure* (see section 3.4.8.2 for further discussion).

3.5.6 Potency of PAH Relative to B[a]P in Rodents

Section 3.5.6 determines the potency of PAH relative to B[a]P in several rodent assays. We have selected data from the mouse skin (see section 3.3.2.2.3) and the rat lung assays (see section 3.3.2.2.4) for this purpose. The results from the mouse skin data could not be used directly, because the duration of the experiments differed from study to study (see section 3.5.6.1). Therefore we have to select a standard duration to facilitate the comparison of responses from each of the mouse skin assays. Our first step was to set a standard time and estimate the magnitude of response at the standard time for each study (described in section 3.5.6.1).

Next, we used the standardized mouse skin and rat lung data (i.e. responses at the standard time) and developed dose-response curves for each PAH and expressed the risk of each PAH in terms of B[a]P equivalents (described in section 3.5.6.2).

Finally, we examined if there was a good correlation between the potencies derived from the four rodent assays. Once the correlation was confirmed, we combined the results to create a single set of potency estimates for PAH relative to B[a]P in rodents (see sections 3.4.6.1 and 3.4.6.2).

3.5.6.1 Estimating Tumor Response at Standardized Time of Observation

Section 3.3.2.2.3 summarizes the data that have been extracted for three mouse skin tumor assays. The data were derived from a number of studies but the assay conditions were comparable except for the experimental duration. In order to compare or combine data from different reports, it was necessary to estimate potency at a time of observation that would be standardized for each assay. The purpose of this section is to estimate the magnitude of response at the standardized time for each dose level of each PAH in a given assay.

The process of standardizing the time consisted of setting the standard time of observation for each assay (see section 3.5.6.1.1) and developing a process to estimate the magnitude of response at the standard observation time from the magnitude of response at the experimental observation time (see sections 3.5.6.1.2 and 3.5.6.1.3).

3.5.6.1.1 Setting Standard Time of Observation

We selected the standard time of observation for each assay based on several criteria.

- Adjustment is required for the minimum number of experiments.

- The number of experiments that gave a maximal response is roughly comparable to the number of experiments that gave a minimal response at the selected time of observation.

Table 3.5.6.1.1.1 presents the standard time (the time since the animals received their first treatment) we have selected for observation for each of the rodent assays. In one-third or more of the cases, the actual observations were made at the selected standard time during the experiment. About two-thirds or more of the experiments conducted the actual observations within 5 weeks of the selected standard time. The second criterion is necessary to assure a maximum number of data points with measurable response at the selected standard time. If too early a time was chosen, then all responses at the standard time would be low and close to zero. If too long a time (a time when most responses reach the maximum) was chosen, then all responses would be close to maximum at the standard time. No meaningful comparison would be possible.

Table 3.5.6.1.1.1 Standard times selected for each experimental protocol.

Assay Type	Standard Exper. Duration	% Studies that Reported Observation at the Time Indicated		
		Standard	Standard ± 1 w	Standard ± 5 w
CD-1 tumor init. Assay	31 w	32	37	70
SENCAR tumor init. Assay	16 w	48	61	64
C57BL compl. carc. Assay	60 w	78	93	93

3.5.6.1.2 Developing Time-Course Function

Our purpose is to estimate the magnitude of tumor response at a preselected standard time from a known magnitude of response at some other time. Visually, the time course for tumor response in terms of tumor incidence was consistently S-shaped, while multiplicity had a time course of a straight line preceded by a lag period. Since the time course relationship for incidence data was not linear, we need to develop a function in order to translate the magnitude of response from the observed times to standard times.

We selected the Weibull survivor function to estimate the relationship between the duration of experiment and tumor incidence for two main reasons. Firstly, the Weibull survivor model is commonly used in biomedical research in studies on time and tumor occurrence both in humans and animals (Lawless, 1982). Secondly, the model (sometimes in a modified form) is often used in risk assessment (Pike, 1966; USEPA, 1991; Clement Associates, 1990).

Equation 3.5.6.1.2.1 describes the Weibull Model we used for the exercise.

$$S(t) = \exp\{-(\lambda t)^{\beta}\}$$ Equation 3.5.6.1.2.1

where S(t) is the fraction of tumor-free mice at time, t
λ and β are parameters to be estimated
t is the time in weeks

The Weibull Model we selected usually generates an "S"-shaped curve and the parameter β is called the "shape parameter" (Lawless, 1982). In order to evaluate the values for the two parameters, λ and β, that fit our tumorigenicity data the best, we transformed equation 3.5.6.1.2.1 to Equation 3.5.6.1.2.2.

$$\ln\{-lnS(t)\} = \alpha + \beta lnt$$ Equation 3.5.6.1.2.2

where $\alpha = \beta \ln \lambda$

Equation 3.5.6.1.2.2 is linear and the value of β can be estimated by linear regression on the data points. We used the time course data to determine the average value of the parameter ß for each of the three mouse skin assays (results presented in table 3.5.6.1.2.1).

Table 3.5.6.1.2.1 ß parameter for the Weibull function designed to fit the time course of three mouse skin assays.

Mouse Strain / Assay Type	"Shape" (ß)
CD-1 tumor initiation assay	2
SENCAR tumor initiation assay	2
C57BL complete carc. Assay	4.5

We assessed how well the Weibull function fitted the experimental data by examining the correlation coefficient (r) and the p-values for the linear relationship between $\ln\{-lnS(t)\}$ and lnt (Note that the p-value is an indicator of the probability that no correlation exists and

that any apparent correlation results from chance). The goodness-of-fit was also tested by graphing the observed data with the Weibull model. Visual inspection of the graphs agreed with the correlation test.

The results of the correlation are shown in table 3.5.6.1.2.2. On average, the fit of the Weibull function to the experimental tumor incidence data is excellent in 86% of the cases according to our definition for *excellent*, *good*, and *poor fit* (column two, table 3.5.6.1.2.2). In about 10% of the cases, the fit is poor. Among the three assays, the data from the CD-1 mice tumor initiation assay and C57BL mice complete carcinogenicity assay fit the Weibull function very well. The data for the SENCAR mice fit acceptably, although not as well.

Table 3.5.6.1.2.2 Percentage of experiments categorized according to goodness-of-fit to the Weibull function in each essay.

Category of Fit	Definition of Category	CD-1 Assay	SENCAR Assay	C57BL Assay	Average of Three Assays
Excellent	$p \leq 0.05$ and $r^2 \geq 0.64$	89%	55%	95%	86%
Good	$p > 0.05$ or $r^2 < 0.64$	6%	9%	0%	5%
Poor	$p > 0.05$ and $r^2 < 0.64$	6%	36%	5%	10%

We therefore applied the Weibull function to estimate the tumor response at the standard time for all tumor incidence experiments (standard times for each assay type are contained in table 3.5.6.1.1). Details are provided in section 3.5.6.1.3.

3.5.6.1.3 Estimating Tumor Incidence Response at Standard Time

We extrapolated the magnitude of tumor incidence response at the standard time from the magnitude of response at the time of observation at each PAH dose level for each experiment. The extrapolation consists of the following steps.

- Obtain the magnitude of response and the experimental duration from the mouse skin tumorigenicity database (see section 3.3.2.2.3).
- Substitute the values of tumor incidence, duration of experiment and ß parameter (from table 3.5.6.1.2.1) into equation 3.5.6.1.2.2 to calculate the value for the α parameter.

- Use equation 3.5.6.1.2.2 and the values of the standard experimental duration for the particular assay, α and β parameters to estimate the magnitude of response at the standard time.
- For the complete carcinogenicity assay using C57BL mice, the standardization of experimental duration requires an additional adjustment to the dose. In the tumor initiation experiments, PAH is administered at the onset of the experiment only. As a result, a change in the experimental duration changes the dose of TPA only and not the total dose of PAH administered. In contrast, PAH are administered throughout the course of the complete carcinogenicity experiment. Thus the total dose of PAH administered changes with the duration of the experiment. We therefore have to adjust the total dose of PAH to account for the difference in the total dose administered at standard duration and at the experimental duration.

In order to validate the time standardization procedure, the observed responses and the responses predicted from the Weibull function were plotted and compared. A detailed inspection of the data led us to conclude that the time standardization method predicted the time-course of tumor development very well. For example, a given PAH, tested at a given dose in a given assay (e.g. CD-1, SENCAR, C57BL) was found to produce a consistent response at the standard time, independent of the laboratories that conducted the experiments.

3.5.6.1.4 Estimating Tumor Multiplicity Response at Standard Time

Tumor multiplicity data appear to have a different time-course relationship than tumor incidence data. The relationship between the time of observation and the magnitude of response appeared to be linear, but there was a lag period during which no tumors were observed regardless of the dose. On inspection, the duration of the lag period seemed to be relatively independent of the dose. We used the linear regression procedure to establish the relationship between the duration of the experiment and the magnitude of tumor multiplicity response. The intercept on the x-axis gave the value for the duration of the lag period for each experiment. The median value for the x-intercept from all available time-course data sets was used in the estimation of the magnitude of tumor multiplicity response at standard times at each dose level for PAH using equation 3.5.6.1.4.1. Table 3.5.6.1.4.1 summarizes the values for the standard durations ($Time_{std}$) and the duration of the lag period ($Time_{lag}$) used for the calculations.

$$No\_Of\_Tumors_{std} = \left[\frac{No\_Of\_Tumors_{obs}}{Time_{obs} - Time_{lag}} \right] (Time_{std} - Time_{obs}) + No\_Of\_Tumors_{obs}$$

Equation 3.5.6.1.4.1

Table 3.5.6.1.4.1 Constants for determination of tumor multiplicity at standard times.

Rodent Assay	Lag Period (weeks)	Standard Time (weeks)
CD-1 mice	7	31
SENCAR mice	8	16

3.5.6.2 Estimating Potency of PAH in Four Rodent Assays

The mouse data adjusted for standardized experimental duration and the rat lung data (see section 3.3.2.2.4) were used to establish the dose-response curves. Within each assay, the data for each PAH from different laboratories were combined, since studies conducted in different laboratories have been shown to provide comparable results. We used both the tumor incidence and multiplicity data, and several methods to estimate the potency of PAH.

We have considered the following dose-response mathematical functions to fit the incidence data.

- **Log probit function**

  The log-probit function is commonly used to assess dose-response relationships in both pharmacological and toxicological bioassays (Endrenyi, 1989; Grimmer *et al.* 1984, 1987). The probit function is based on the transformation of the incidence to a probit scale using a cumulative normal distribution (see the following equation).

  $$\Phi(z) = \int_{-\infty}^{z} \frac{1}{\sqrt{2\pi}} e^{\left(-\frac{u^2}{2}\right)} du$$

  When the probit model fits the data, the plot of the probit (i.e. transformed incidence) versus log (dose) forms a straight line. However, the log probit function fitted our data poorly and the function was not considered further.

- **Linearized multistage model**

  The linearized multistage model was developed as a biologically based function to model carcinogenicity. One of the main assumptions underlying the model is that the slope is thought to be linear in the low dose region of the dose-response curve (Anderson, 1983). The following is the equation for the multistage model.

  $$P(d) = 1 - e^{\left(-q_0 - q_1 d - q_2 d^2 \ldots - q_k d^k\right)}$$

  where P(d) represents the lifetime risk of cancer at dose d; $q_i > 0$; i = 0,1,2,...,k and k = (number of doses - 1). The point estimates of the coefficients (i.e. q_i) were obtained by fitting the data using the software program TOX_RISK. The q_1 parameter is the linear coefficient and q_1* is the 95% upper confidence limit for this parameter.

  We considered the linearized multistage model because the model has been widely used by US EPA and by many risk assessors (see table 3.3.1.1). However, the model did not fit some of our data well. In particular, it failed when the dose-response data consisted of a low incidence in the control group and a maximal or close to maximal incidence in the treated groups. The failure was considered to be a serious drawback because it resulted in very low potency estimates for some very potent compounds even though inspection of the data suggested maximal or submaximal responses at low doses. The failure to predict tumor potency applied to compounds that were tested only at doses which produced a very high incidence rate. Therefore, we decided not to use the linearized multistage model.

- **Linear regression**

  Since none of the more sophisticated dose-response models fitted the data particularly well, we have used linear regression as the primary means of estimating PAH potency. In some instances, the compounds induced maximal or *submaximal* responses at several consecutive doses. Submaximal doses are defined for the purpose of this assessment as doses which induce a tumor response in at least 80% of the treated mice. In determining the initial slope, we removed from the data points subsequent (sub)maximal dose(s) which would reduce the slope estimate. For those PAH for which only two points were available, we calculated the slope of the straight line through the points in terms of incidence/mg. When the lowest administered dose induced maximal response, our estimate is a lower bound of the actual potency. The actual potency is equal to or higher than the calculated value.

For multiplicity data, we considered the following functions.

- **$ED_{0.10}$ using Negative Binomial Model (NBM)**

  The Negative Binomial Model was used to model the relationship between dose (in mg) and response (measured as average number of tumors per mouse). The model accounts for the variation in the appearance of tumors within an animal, as well as animal-to-animal variability in sensitivity (Lewtas, personal communication). The function is shown below:

  $$m_i = \beta_0 + e^{(\beta_1 + \beta_2 \ln(d_i))}$$

  where m_i is the average number of tumors per mouse for dose group, d_i.

  Since the parameter estimates are difficult to interpret, the dose expected to produce 0.10 tumors per mouse ($ED_{0.10}$) was calculated by rearranging the above formula and substituting m_i=0.10. Thus, a more potent compound produces an effect at a lower dose than a less potent compound.

- **Linear regression**

  Since the NBM did not fit the data well, we used the linear regression process to estimate the tumor potency for PAH, expressed in terms of multiplicity per mg of PAH. Since the slope of the dose-response curve tends to decrease at high doses (on saturation), we have excluded those high dose points which would reduce the estimate of the initial slope of the dose-response curve. For those PAH for which only two points were available, we have calculated the incidence/mg PAH.

After evaluating the suitability of the various models for fitting PAH data, we selected linear regression as a means to estimate tumorigenic potency for both the incidence and multiplicity data sets. Linear regression method was chosen because of its simplicity and because the more complex models did not fit the data any better.

Although human risk is usually not expressed in terms of tumor multiplicity, but rather tumor incidence, tumor multiplicity is sometimes preferred in reporting experimental animal data. When tumor incidence is used as an index of tumorigenicity, dose-dependence for a given PAH can be demonstrated only within a limited dose-range . If the dose is too low, there is no tumorigenic effect. If the dose is too high, the dose becomes supramaximal in that it produces a maximum response identical to a somewhat lower dose. In contrast, with tumor multiplicity, increase in response can be accurate up to about three to four tumors per mouse. Beyond that, it is difficult to distinguish between individual tumors in an animal (D. Jerina, private com-

munication). The dose range within which dose-dependence of tumor multiplicity in an animal can be observed tends to be wider than the dose range within which dose-dependence of tumor incidence can be observed.

We prefer to use the incidence data rather than the multiplicity data, because it does not require making the assumption that the occurrence of the second tumor is independent of the presence of the first tumor. Furthermore, use of tumor incidence allows us to use the same measure of tumorigenicity in both experimental animals and humans. We therefore compared the potency of individual PAH measured in terms of tumor incidence with that expressed in terms of tumor multiplicity. There was a strong correlation between the two estimates (CD-1: Pearson's $r = 0.752$, $p = 0.0001$, $n = 154$; SENCAR: Pearson's $r = 0.926$, $p = 0.0001$, $n = 54$).

The correlation serves two purposes. First, the comparison result confirms that there is indeed a strong correlation between the two measures of tumorigenicity: tumor incidence and multiplicity. Perhaps more importantly, the good correlation result is indicative that our time normalization has been appropriate. Despite the use of different time normalization processes and distinct (but not independent) data sets in estimating the potency of PAH for the two different expressions of tumorigenicity (see section 3.5.6.1), the correlation between the potencies for different expression of tumorigenicity in both assays is good.

We have also conducted similar comparison between the dose-response curves derived using the linearized multistage model and linear regression. The two measures of tumorigenic potency correlated well with each other (especially for the CD-1 and C57BL bioassays).

The potencies of individual PAH derived from the four rodent assays were used to develop the relative potency of individual PAH (relative to B[a]P). The process of developing relative potency for PAH is described in section 3.5.6.3.

3.5.6.3 Estimating Potency of PAH Relative to B[a]P in Rodents

We have taken the following steps to estimate the relative potency of PAH to B[a]P.

- We estimated the potencies of PAH relative to B[a]P in the four rodent assays using the data described in section 3.5.6.2. The relative potency of a PAH was calculated as the ratio of the potency of the PAH to the potency of B[a]P determined from the assay.
- On validating that the relative potency of PAH was similar in the four assays (see sections 3.4.6.1 and 3.4.6.2), we combined the four data sets.

- When the relative potency was available in more than one assay, only one data set was used. Selection of the data set was conducted in the following decreasing order of preference.
  - tumor initiation assay with CD-1 mice, being the largest data set.
  - tumor initiation assay with SENCAR mice, being the next largest data set whose potencies correlated best with the potencies from the CD-1 assay (see section 3.4.6.1).
  - rat lung assay, with a number of environmentally relevant PAH tested in the assay.
  - complete carcinogenicity assay on C57BL mice, with excellent correlation with the tumor initiation assay with CD-1 mice, but most of the compounds tested in this assay were also tested in the CD-1 assay.

The relative potencies estimated for individual PAH using the methods described are summarized in table 3.5.7.1.

3.5.7 Human Cancer Risk from Exposure to PAH by Different Routes and at Different Organ Sites

Section 3.5.7 estimates the lifetime lung, stomach and skin cancer risks in humans from exposure to a variety of PAH by the oral, inhalation and dermal routes.

The route- and site-specific lifetime cancer risks from exposure to individual PAH in humans were calculated by multiplying the site- and route-specific risk estimate for B[a]P (from table 3.5.5.1) by the relative potencies of the PAH to B[a]P (from the third column in table 3.5.7.1). The human potency estimate for each PAH exposure route and at each tumor site is a product of the B[a]P potency at the same site and for the same exposure route, and the relative potency of the given PAH to B[a]P. The relationship can be expressed by the following equation.

Human potency of $PAH_{site,\ route\ of\ exposure}$

= Relative potency of PAH to B[a]P x Human potency of $B[a]P_{site,\ route\ of\ exposure}$

The results are summarized in Table 3.5.7.1.

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
acridine	000260-94-6	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
anthanthrene	000191-26-4	2.8E-01	2.5E-07	5.9E-11	3.9E-08	7.4E-10	3.4E-08	4.1E-07	6.6E-08
benz[a]anthracene,10,11-dihydroxy-10,11-dihydro-7-(hydroxymethyl)-12-methyl-,trans-,(+/-)	071722-00-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene	000056-55-3	1.4E-02	1.2E-08	3.0E-12	2.0E-09	3.7E-11	1.7E-09	2.1E-08	3.3E-09
benz[a]anthracene,1,2,3,4-tetrahydro-7,12-dimethyl-,	067242-54-0	5.5E+00	4.8E-06	1.2E-09	7.7E-07	1.5E-08	6.8E-07	8.1E-06	1.3E-06
benz[a]anthracene,1,2-dihydro-	060968-08-3	1.8E-02	1.6E-08	3.8E-12	2.5E-09	4.7E-11	2.2E-09	2.7E-08	4.3E-09
benz[a]anthracene,1,2-dihydroxy-1,2-dihydro-,trans-,(+/-)	064807-77-8	6.8E-03	6.0E-09	1.4E-12	9.6E-10	1.8E-11	8.4E-10	1.0E-08	1.6E-09
benz[a]anthracene,1,2-dihydroxy-1,2-dihydro-8-(hydroxymethyl)-,trans-	073195-10-5	4.6E-02	4.0E-08	9.7E-12	6.5E-09	1.2E-10	5.7E-09	6.8E-08	1.1E-08
benz[a]anthracene,1,7,12-trimethyl-,	060786-51-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,1-fluoro-7,12-dimethyl-,	068141-57-1	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,1-hydroxy-7,12-dimethyl-,	066240-13-9	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,10,11-dihydroxy-,10,11-dihydro-,trans-,(+/-)	060967-90-0	5.6E-03	4.9E-09	1.2E-12	7.9E-10	1.5E-11	6.9E-10	8.3E-09	1.3E-09
benz[a]anthracene,10,11-dihydroxy-10,11-dihydro-7,12-dimethyl,trans-,(+/-)	092622-79-2	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,10,11-dihydroxy-10,11-dihydro-8-(hydroxymethyl)-,trans,(+/-)	078617-07-9	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,10-hydroxy-7,12-dimethyl-,	062064-38-4	8.2E-02	7.2E-08	1.7E-11	1.2E-08	2.2E-10	1.0E-08	1.2E-07	1.9E-08
benz[a]anthracene,11-fluoro-7,12-dimethyl-,	002023-61-2	3.2E-01	2.8E-07	6.7E-11	4.5E-08	8.4E-10	3.9E-08	4.7E-07	7.5E-08
benz[a]anthracene,12-(bromomethyl)-7-methyl-,	059230-81-8	9.2E-02	8.1E-08	1.9E-11	1.3E-08	2.4E-10	1.1E-08	1.4E-07	2.2E-08
benz[a]anthracene,12-(hydroxymethyl)-7-methyl-,	000568-70-7	2.3E-01	2.0E-07	4.9E-11	3.2E-08	6.1E-10	2.8E-08	3.4E-07	5.4E-08
benz[a]anthracene,12-carbaldehyde-7-methyl-,	017513-40-5	4.4E-02	3.9E-08	9.3E-12	6.2E-09	1.2E-10	5.4E-09	6.5E-08	1.0E-08

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
benz[a]anthracene,2,7,12-trimethyl-,	035215-31-7	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,2-fluoro-7,12-dimethyl-,	068141-56-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,2-hydroxy-7,12-dimethyl-,	066240-31-1	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,3,4-dihydro-,	060968-01-6	1.6E-01	1.4E-07	3.4E-11	2.2E-08	4.2E-10	2.0E-08	2.4E-07	3.8E-08
benz[a]anthracene,3,4-dihydroxy-3,4-dihydro-,trans-,(+/-)	064501-86-6	1.3E-01	1.1E-07	2.7E-11	1.8E-08	3.4E-10	1.6E-08	1.9E-07	3.1E-08
benz[a]anthracene,3,4-dihydroxy-3,4-dihydro-7,12-di(hydroxymethyl)-,(+/-)	100000\00\5	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,3,4-dihydroxy-3,4-dihydro-7,12-dimethyl-,trans-,(+/-)	074938-86-6	1.9E+01	1.7E-05	4.0E-09	2.7E-06	5.0E-08	2.3E-06	2.8E-05	4.5E-06
benz[a]anthracene,3,4-dihydroxy-3,4-dihydro-7-(hydroxymethyl)-12-methyl-,trans-,(+/-)	069260-84-0	1.6E+00	1.4E-06	3.4E-10	2.2E-07	4.2E-09	2.0E-07	2.4E-06	3.8E-07
benz[a]anthracene,3,4-dihydroxy-3,4-dihydro-7-methyl-,trans-,(+/-)	074634-54-1	3.9E-01	3.4E-07	8.2E-11	5.5E-08	1.0E-09	4.8E-08	5.8E-07	9.2E-08
benz[a]anthracene,3,4-dihydroxy-3,4-dihydro-8-(hydroxymethyl)-,trans-	078617-05-7	1.5E-01	1.3E-07	3.2E-11	2.1E-08	4.0E-10	1.8E-08	2.2E-07	3.5E-08
benz[a]anthracene,3,4-dihydroxy-3,4-dihydro-8-methyl-,trans-	094849-77-1	9.8E-01	8.6E-07	2.1E-10	1.4E-07	2.6E-09	1.2E-07	1.4E-06	2.3E-07
benz[a]anthracene,3,9-dimethyl-	000316-51-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,3-hydroxy-7,12-dimethyl-,	057266-83-8	2.7E-02	2.4E-08	5.7E-12	3.8E-09	7.1E-11	3.3E-09	4.0E-08	6.4E-09
benz[a]anthracene,3.α.,4.ß.-dihydroxy-1.α.,2.α.-epoxy-1,2,3,4-tetrahydro-,(+/-)	064598-80-7	3.5E-02	3.1E-08	7.4E-12	4.9E-09	9.2E-11	4.3E-09	5.2E-08	8.3E-09
benz[a]anthracene,3.α.,4.ß.-dihydroxy-1.α.,2.α.-epoxy-7-methyl-1,2,3,4-tetrahydro-,trans-,(+/-)	064625-66-7	6.7E-02	5.9E-08	1.4E-11	9.4E-09	1.8E-10	8.2E-09	9.9E-08	1.6E-08
benz[a]anthracene,3.α.,4.ß.-dihydroxy-1.ß.,2.ß.-epoxy-1,2,3,4-tetrahydro-,(+/-)	064598-81-8	1.5E-01	1.3E-07	3.2E-11	2.1E-08	4.0E-10	1.8E-08	2.2E-07	3.5E-08
benz[a]anthracene,3.α.,4.ß.-dihydroxy-3,4-dihydro-,(-)	067335-42-6	4.4E-01	3.9E-07	9.3E-11	6.2E-08	1.2E-09	5.4E-08	6.5E-07	1.0E-07
benz[a]anthracene,3.ß.,4.α.-dihydroxy-3,4-dihydro-,(+)	067335-43-7	5.2E-02	4.6E-08	1.1E-11	7.3E-09	1.4E-10	6.4E-09	7.7E-08	1.2E-08
benz[a]anthracene,4-hydroxy-7,12-dimethyl-,	014760-53-3	2.7E-02	2.4E-08	5.7E-12	3.8E-09	7.1E-11	3.3E-09	4.0E-08	6.4E-09

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
benz[a]anthracene,5,6-dihydro-5,6-epoxy-7-(hydroxymethyl)-12-methyl-,(+/-)	040495-38-3	1.7E-02	1.5E-08	3.6E-12	2.4E-09	4.5E-11	2.1E-09	2.5E-08	4.0E-09
benz[a]anthracene,5,6-dihydroxy-5,6-dihydro-,trans-,(+/-)	067315-17-7	5.9E-03	5.2E-09	1.2E-12	8.3E-10	1.6E-11	7.3E-10	8.7E-09	1.4E-09
benz[a]anthracene,5,6-dihydroxy-5,6-dihydro-7,12-dimethyl-,trans-,(+/-)	075262-88-3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,5,6-dihydroxy-5,6-dihydro-7-(hydroxymethyl)-12-methyl-,trans-,(+/-)	040495-42-9	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,5,6-dihydroxy-5,6-dihydro-8-(hydroxymethyl)-,trans-	077102-53-5	6.6E-02	5.8E-08	1.4E-11	9.3E-09	1.7E-10	8.1E-09	9.8E-08	1.6E-08
benz[a]anthracene,5,6-dihydroxy-5,6-dihydro-8-methyl-,trans-,(+/-)	094850-06-3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,5,7,12-trimethyl-,	024891-39-2	9.6E-02	8.4E-08	2.0E-11	1.3E-08	2.5E-10	1.2E-08	1.4E-07	2.3E-08
benz[a]anthracene,5,7-dimethyl-,	041385-99-3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,5-fluoro-7,12-dimethyl-,	000794-00-3	3.1E-02	2.7E-08	6.5E-12	4.4E-09	8.2E-11	3.8E-09	4.6E-08	7.3E-09
benz[a]anthracene,5-hydroxy-7,12-dimethyl-,	059056-51-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,6,8-dimethyl-	000317-64-6	1.2E-01	1.1E-07	2.5E-11	1.7E-08	3.2E-10	1.5E-08	1.8E-07	2.8E-08
benz[a]anthracene,7,12-di(hydroxymethyl)-	002564-65-0	1.0E+00	8.8E-07	2.1E-10	1.4E-07	2.6E-09	1.2E-07	1.5E-06	2.4E-07
benz[a]anthracene,7,12-dicarbaldehyde-,	019926-22-8	4.4E-02	3.9E-08	9.3E-12	6.2E-09	1.2E-10	5.4E-09	6.5E-08	1.0E-08
benz[a]anthracene,7,12-dimethyl-,	000057-97-6	1.9E+01	1.7E-05	4.0E-09	2.7E-06	5.0E-08	2.3E-06	2.8E-05	4.5E-06
benz[a]anthracene,7-(bromomethyl)-,	024961-39-5	1.1E-02	9.7E-09	2.3E-12	1.5E-09	2.9E-11	1.4E-09	1.6E-08	2.6E-09
benz[a]anthracene,7-(bromomethyl)-12-methyl-,	016238-56-5	1.0E-01	8.8E-08	2.1E-11	1.4E-08	2.6E-10	1.2E-08	1.5E-07	2.4E-08
benz[a]anthracene,7-(hydroxymethyl)-12-methyl-,	000568-75-2	6.8E-02	6.0E-08	1.4E-11	9.6E-09	1.8E-10	8.4E-09	1.0E-07	1.6E-08
benz[a]anthracene,7-carbaldehyde-12-methyl-,	013345-61-4	7.2E-02	6.3E-08	1.5E-11	1.0E-08	1.9E-10	8.9E-09	1.1E-07	1.7E-08
benz[a]anthracene,7-methyl-,	002541-69-7	3.2E-01	2.8E-07	6.7E-11	4.5E-08	8.4E-10	3.9E-08	4.7E-07	7.6E-08

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
benz[a]anthracene,8,9-dihydroxy-8,9-dihydro-,trans-,(+/-)	067262-34-4	1.1E-02	9.7E-09	2.3E-12	1.5E-09	2.9E-11	1.4E-09	1.6E-08	2.6E-09
benz[a]anthracene,8,9-dihydroxy-8,9-dihydro-7,12-dimethyl-,trans-,(+/-)	074938-87-7	3.5E-02	3.1E-08	7.4E-12	4.9E-09	9.2E-11	4.3E-09	5.2E-08	8.3E-09
benz[a]anthracene,8,9-dihydroxy-8,9-dihydro-7-(hydroxymethyl)-12-methyl-,trans-,(+/-)	070234-58-1	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benz[a]anthracene,8,9-dihydroxy-8,9-dihydro-8-methyl-,trans-	094849-87-1	6.2E-02	5.4E-08	1.3E-11	8.7E-09	1.6E-10	7.6E-09	9.2E-08	1.5E-08
benz[a]anthracene,8-(hydroxymethyl)-	072533-44-9	1.5E-01	1.3E-07	3.2E-11	2.1E-08	4.0E-10	1.8E-08	2.2E-07	3.5E-08
benz[a]anthracene,8-hydroxy-,	034501-23-0	1.4E-01	1.2E-07	3.0E-11	2.0E-08	3.7E-10	1.7E-08	2.1E-07	3.3E-08
benz[a]anthracene,8-methyl-,	002381-31-9	8.5E-02	7.5E-08	1.8E-11	1.2E-08	2.2E-10	1.0E-08	1.3E-07	2.0E-08
benz[a]anthracene,8.α.,9.ß.-dihydroxy-10.ß.,11.ß.-epoxy-8,9,10,11-tetrahydro-,(+/-)	064598-83-0	1.5E-02	1.3E-08	3.2E-12	2.1E-09	4.0E-11	1.8E-09	2.2E-08	3.5E-09
benz[a]anthracene,9-hydroxy-7,12-dimethyl-,	066240-06-0	6.2E-02	5.4E-08	1.3E-11	8.7E-09	1.6E-10	7.6E-09	9.2E-08	1.5E-08
benz[e]aceanthrylene	000199-54-2	1.2E-01	1.1E-07	2.5E-11	1.7E-08	3.2E-10	1.5E-08	1.8E-07	2.8E-08
benz[l]aceanthrylene	000211-91-6	7.7E-01	6.8E-07	1.6E-10	1.1E-07	2.0E-09	9.5E-08	1.1E-06	1.8E-07
benzo[a]acridine	000225-11-6	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene	000050-32-8	1.0E+00	8.8E-07	2.1E-10	1.4E-07	2.6E-09	1.2E-07	1.5E-06	2.4E-07
benzo[a]pyrene,1,2-dimethyl-,	016757-85-0	2.8E-01	2.5E-07	5.9E-11	3.9E-08	7.4E-10	3.4E-08	4.1E-07	6.6E-08
benzo[a]pyrene,1,6-dimethyl-,	016757-90-7	1.4E-01	1.2E-07	3.0E-11	2.0E-08	3.7E-10	1.7E-08	2.1E-07	3.3E-08
benzo[a]pyrene,1,6-quinone-,	003067-13-8	5.0E-02	4.4E-08	1.1E-11	7.0E-09	1.3E-10	6.2E-09	7.4E-08	1.2E-08
benzo[a]pyrene,1-hydroxy-,	013345-23-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,1-methyl-,	040568-90-9	6.2E-01	5.4E-07	1.3E-10	8.7E-08	1.6E-09	7.6E-08	9.2E-07	1.5E-07
benzo[a]pyrene,10-hydroxy-,	056892-31-0	2.2E-02	1.9E-08	4.6E-12	3.1E-09	5.8E-11	2.7E-09	3.3E-08	5.2E-09

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
benzo[a]pyrene,10-methyl-,	063104-32-5	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,11,12-dihydro-11,12-epoxy-,(+/-)	067445-96-9	1.1E-01	9.7E-08	2.3E-11	1.5E-08	2.9E-10	1.4E-08	1.6E-07	2.6E-08
benzo[a]pyrene,11-hydroxy-,	056892-32-1	1.3E-01	1.1E-07	2.7E-11	1.8E-08	3.4E-10	1.6E-08	1.9E-07	3.1E-08
benzo[a]pyrene,11-methyl-,	016757-80-5	7.0E-01	6.2E-07	1.5E-10	9.8E-08	1.8E-09	8.6E-08	1.0E-06	1.7E-07
benzo[a]pyrene,12-hydroxy-,	056892-33-2	5.1E-02	4.5E-08	1.1E-11	7.2E-09	1.3E-10	6.3E-09	7.5E-08	1.2E-08
benzo[a]pyrene,12-methyl-,	004514-19-6	5.3E-01	4.7E-07	1.1E-10	7.5E-08	1.4E-09	6.5E-08	7.8E-07	1.3E-07
benzo[a]pyrene,2-hydroxy-,	056892-30-9	1.4E-01	1.2E-07	3.0E-11	2.0E-08	3.7E-10	1.7E-08	2.1E-07	3.3E-08
benzo[a]pyrene,2-methyl-,	016757-82-7	2.7E-01	2.4E-07	5.7E-11	3.8E-08	7.1E-10	3.3E-08	4.0E-07	6.4E-08
benzo[a]pyrene,3,6-dimethyl-,	016757-91-8	2.8E-01	2.5E-07	5.9E-11	3.9E-08	7.4E-10	3.4E-08	4.1E-07	6.6E-08
benzo[a]pyrene,3,6-quinone-,	003067-14-9	3.6E-02	3.2E-08	7.6E-12	5.1E-09	9.5E-11	4.4E-09	5.3E-08	8.5E-09
benzo[a]pyrene,3-hydroxy-,	013345-21-6	2.4E-02	2.1E-08	5.1E-12	3.4E-09	6.3E-11	3.0E-09	3.5E-08	5.7E-09
benzo[a]pyrene,3-methyl-,	016757-81-6	5.9E-01	5.2E-07	1.2E-10	8.3E-08	1.6E-09	7.3E-08	8.7E-07	1.4E-07
benzo[a]pyrene,4,5-dihydro-4,5-epoxy-,(+/-)	064437-52-1	7.2E-02	6.3E-08	1.5E-11	1.0E-08	1.9E-10	8.9E-09	1.1E-07	1.7E-08
benzo[a]pyrene,4,5-dimethyl-,	016757-89-4	4.5E-01	4.0E-07	9.5E-11	6.3E-08	1.2E-09	5.5E-08	6.7E-07	1.1E-07
benzo[a]pyrene,4-hydroxy-,	037574-48-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,4-methyl-,	016757-83-8	5.1E-01	4.5E-07	1.1E-10	7.2E-08	1.3E-09	6.3E-08	7.5E-07	1.2E-07
benzo[a]pyrene,5-hydroxy-,	024027-84-7	2.2E-02	1.9E-08	4.6E-12	3.1E-09	5.8E-11	2.7E-09	3.3E-08	5.2E-09
benzo[a]pyrene,5-methyl-,	031647-36-6	1.8E-01	1.6E-07	3.8E-11	2.5E-08	4.7E-10	2.2E-08	2.7E-07	4.3E-08
benzo[a]pyrene,6,12-quinone-,	003067-12-7	5.0E-02	4.4E-08	1.1E-11	7.0E-09	1.3E-10	6.2E-09	7.4E-08	1.2E-08

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
benzo[a]pyrene,6-(hydroxymethyl)-,	021247-98-3	8.7E-02	7.6E-08	1.8E-11	1.2E-08	2.3E-10	1.1E-08	1.3E-07	2.1E-08
benzo[a]pyrene,6-hydroxy-,	033953-73-0	2.9E-02	2.5E-08	6.1E-12	4.1E-09	7.6E-11	3.6E-09	4.3E-08	6.9E-09
benzo[a]pyrene,6-methyl-,	002381-39-7	1.1E-01	9.7E-08	2.3E-11	1.5E-08	2.9E-10	1.4E-08	1.6E-07	2.6E-08
benzo[a]pyrene,7,8-dihydro-7,8-dihydroxy-,(-)	06R007-98-2	7.2E-02	6.3E-08	1.5E-11	1.0E-08	1.9E-10	8.9E-09	1.1E-07	1.7E-08
benzo[a]pyrene,7,8-dihydro-7,8-epoxy-,(+)-	068906-82-1	1.2E-01	1.1E-07	2.5E-11	1.7E-08	3.2E-10	1.5E-08	1.8E-07	2.8E-08
benzo[a]pyrene,7,8-dihydro-7,8-epoxy-,(+/-)	064437-54-3	4.5E-01	4.0E-07	9.5E-11	6.3E-08	1.2E-09	5.5E-08	6.7E-07	1.1E-07
benzo[a]pyrene,7,8-dihydroxy-7,8,9,10-tetrahydro-,trans-,(+/-)	064314-00-7	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7,8-dihydroxy-7,8-dihydro-,trans-,(+/-)	061443-57-0	5.2E-01	4.6E-07	1.1E-10	7.3E-08	1.4E-09	6.4E-08	7.7E-07	1.2E-07
benzo[a]pyrene,7,8-dihydroxy-7,8-dihydro-9-methyl-,trans-,(+/-)	073998-81-9	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7,8-epoxy-7,8,9,10-tetrahydro-,(+/-)	036504-67-3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7-hydroxy-,	037994-82-4	2.9E-02	2.5E-08	6.1E-12	4.1E-09	7.6E-11	3.6E-09	4.3E-08	6.9E-09
benzo[a]pyrene,7-methyl-,	063041-77-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7.α.,8.ß.,-dihydroxy-9.α.,10.α.-epoxy-7,8,9,10-tetrahydro-,(+/-)	058917-91-2	0.0E+00	0.0E+00	0.0E+00	0.0E+ 0	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7.α.,8.ß.-dihydroxy-7,8-dihydro-,(+)	062314-67-4	2.2E-01	1.9E-07	4.6E-11	3.1E-08	5.8E-10	2.7E-08	3.3E-07	5.2E-08
benzo[a]pyrene,7.α.,8.ß.-dihydroxy-9.α.,10.α.-epoxy-7,8,9,10-tetrahydro-,(+/-)	063323-29-5	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7.α.,8.ß.-dihydroxy-9.ß.,10.ß.-epoxy-7,8,9,10-tetrahydro-,(+/-)	058917-67-2	2.0E-01	1.8E-07	4.2E-11	2.8E-08	5.3E-10	2.5E-08	3.0E-07	4.7E-08
benzo[a]pyrene,7.α.,8.ß.-dihydroxy-9.ß.,10.ß.-epoxy-7,8,9,10-tetrahydro-,(-)	063323-30-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7.ß.,8.α.,9.α.,10.α.-tetrahydroxy-7,8,9,10-tetrahydro-,(+/-)	110000\08\0	1.4E-01	1.2E-07	3.0E-11	2.0E-08	3.7E-10	1.7E-08	2.1E-07	3.3E-08
benzo[a]pyrene,7.ß.,8.α.,9.ß.,10.ß.-tetrahydroxy-7,8,9,10-tetrahydro-,(+/-)	110000\07\9	8.5E-02	7.5E-08	1.8E-11	1.2E-08	2.2E-10	1.0E-08	1.3E-07	2.0E-08

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
benzo[a]pyrene,7.ß.,8.α.-dihydroxy-9.α.,10.α.-epoxy-7,8,9,10-tetrahydro-,(+)	063323-31-9	3.6E-01	3.2E-07	7.6E-11	5.1E-08	9.5E-10	4.4E-08	5.3E-07	8.5E-08
benzo[a]pyrene,7.ß.,8.α.-dihydroxy-9.ß.,10.ß.-epoxy-7,8,9,10-tetrahydro-,(-)	063357-09-5	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,7.ß.,8.α.dihydroxy-7,8-dihydro,(-)	060864-95-1	1.2E+00	1.1E-06	2.5E-10	1.7E-07	3.2E-09	1.5E-07	1.8E-06	2.8E-07
benzo[a]pyrene,8-hydroxy-,	013345-26-1	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,8-methyl-,	063041-76-9	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[a]pyrene,9,10-dihydro-9,10-epoxy-,(+/-)	064437-53-2	3.0E-02	2.6E-08	6.3E-12	4.2E-09	7.9E-11	3.7E-09	4.4E-08	7.1E-09
benzo[a]pyrene,9,10-dihydroxy-9,10-dihydro-,trans-,(+/-)	058030-91-4	1.3E-02	1.1E-08	2.7E-12	1.8E-09	3.4E-11	1.6E-09	1.9E-08	3.1E-09
benzo[a]pyrene,9,10-epoxy-7,8,9,10-tetrahydro-,(+/-)	064608-56-6	8.9E-03	7.8E-09	1.9E-12	1.3E-09	2.3E-11	1.1E-09	1.3E-08	2.1E-09
benzo[a]pyrene,9-hydroxy-,	017573-21-6	5.1E-02	4.5E-08	1.1E-11	7.2E-09	1.3E-10	6.3E-09	7.5E-08	1.2E-08
benzo[a]pyrene,9-methyl-,	070644-19-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[b]fluoranthene	000205-99-2	1.1E-01	9.7E-08	2.3E-11	1.5E-08	2.9E-10	1.4E-08	1.6E-07	2.6E-08
benzo[b]naphtho[2,1-d]thiophene	000239-35-0	2.8E-02	2.5E-08	5.9E-12	3.9E-09	7.4E-11	3.4E-09	4.1E-08	6.6E-09
benzo[b]triphenylene	000215-58-7	1.4E-02	1.2E-08	3.0E-12	2.0E-09	3.7E-11	1.7E-09	2.1E-08	3.3E-09
benzo[c]acridine	000225-51-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[c]phenanthrene	000195-19-7	2.3E-02	2.0E-08	4.9E-12	3.2E-09	6.1E-11	2.8E-09	3.4E-08	5.4E-09
benzo[c]phenanthrene,1,2-dihydro-,	073093-16-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[c]phenanthrene,1,2-dihydroxy-1,2-dihydro-,trans-,(+/-)	073093-22-8	2.50-03	2.2E-09	5.3E-13	3.5E-10	6.6E-12	3.1E-10	3.7E-09	5.9E-10
benzo[c]phenanthrene,3,4-dihydroxy-1,2,3,4-tetrahydro-,trans-,(+/-)	086602-29-1	7.2E-03	6.3E-09	1.5E-12	1.0E-09	1.9E-11	8.9E-10	1.1E-08	1.7E-09
benzo[c]phenanthrene,3,4-dihydroxy-3,4-dihydro-,trans-,(+/-)	079117-84-3	2.8E-02	2.5E-08	5.9E-12	3.9E-09	7.4E-11	3.4E-09	4.1E-08	6.6E-09

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
benzo[c]phenanthrene,3.α.,4.ß.-dihydroxy-1.α.,2.α.-epoxy-1,2,3,4-tetrahydro-,(+/-)	075410-89-8	1.7E-01	1.5E-07	3.6E-11	2.4E-08	4.5E-10	2.1E-08	2.5E-07	4.0E-08
benzo[c]phenanthrene,3.α.,4.ß.-dihydroxy-1.ß.,2.ß.-epoxy-1,2,3,4-tetrahydro-,(+/-)	075443-72-0	1.7E-01	1.5E-07	3.6E-11	2.4E-08	4.5E-10	2.1E-08	2.5E-07	4.0E-08
benzo[c]phenanthrene,5,6-dihydroxy-5,6-dihydro-,trans-,(+/-)	101313-28-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[e]pyrene	∩~0192-97-2	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[e]pyrene,4,5-dihydroxy-4,5-dihydro-,trans-,(+/-)	066788-09-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[e]pyrene,9,10-dihydro-,	066788-01-0	1.4E-02	1.2E-08	3.0E-12	2.0E-09	3.7E-11	1.7E-09	2.1E-08	3.3E-09
benzo[e]pyrene,9,10-dihydroxy-11,12-epoxy-9,10,11,12-tetrahydro-,(+/-)	071213-91-7	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[e]pyrene,9,10-dihydroxy-9,10,11,12-tetrahydro-,trans-,(+/-)	066788-03-2	9.6E-04	8.4E-10	2.0E-13	1.3E-10	2.5E-12	1.2E-10	1.4E-09	2.3E-10
benzo[e]pyrene,9,10-dihydroxy-9,10-dihydro-,trans-,(+/-)	074634-55-2	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
benzo[ghi]perylene	000191-24-2	1.2E-02	1.1E-08	2.5E-12	1.7E-09	3.2E-11	1.5E-09	1.8E-08	2.8E-09
benzo[j]fluoranthene	000205-82-3	4.5E-02	4.0E-08	9.5E-12	6.3E-09	1.2E-10	5.5E-09	6.7E-08	1.1E-08
benzo[k]fluoranthene	000207-08-9	3.7E-02	3.3E-08	7.8E-12	5.2E-09	9.8E-11	4.6E-09	5.5E-08	8.8E-09
benzo[rst]pentaphene	000189-55-9	1.1E+00	9.7E-07	2.3E-10	1.5E-07	2.9E-09	1.4E-07	1.6E-06	2.6E-07
benzo[rst]pentaphene,3,4-dihydroxy-1,2,3,4-tetrahydro-,trans-,(+/-)	080648-54-0	9.7E-01	8.5E-07	2.0E-10	1.4E-07	2.6E-09	1.2E-07	1.4E-06	2.3E-07
benzo[rst]pentaphene,3,4-dihydroxy-3,4-dihydro-,trans-,(+/-)	073691-53-9	1.0E+00	8.8E-07	2.1E-10	1.4E-07	2.6E-09	1.2E-07	1.5E-06	2.4E-07
benzo[rst]pentaphene,3.α.,4.ß.-dihydroxy-1.α.,2.α.-epoxy-1,2,3,4-tetrahydro-,(+/-)	078919-12-7	9.4E-01	8.3E-07	2.0E-10	1.3E-07	2.5E-09	1.2E-07	1.4E-06	2.2E-07
cholanthren-1-one,3-methyl-	003343-07-5	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
cholanthrene,1-hydroxy-3-methyl-,(+/-)	065799-04-4	5.9E-01	5.2E-07	1.2E-10	8.3E-08	1.6E-09	7.3E-08	8.7E-07	1.4E-07
cholanthrene,11,12-dihydroxy-11,12-dihydro-3-methyl-,trans-,(+/-)	105967-38-2	3.8E-01	3.3E-07	8.0E-11	5.3E-08	1.0E-09	4.7E-08	5.6E-07	9.0E-08

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
cholanthrene,2-hydroxy-3-methyl-,(+/-)	091484-84-3	9.8E+00	8.6E-06	2.1E-09	1.4E-06	2.6E-08	1.2E-06	1.4E-05	2.3E-06
cholanthrene,3,11-dimethyl-,	071765-96-3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
cholanthrene,3-methyl-,	000056-49-5	2.6E+00	2.3E-06	5.5E-10	3.7E-07	6.9E-09	3.2E-07	3.8E-06	6.2E-07
cholanthrene,9,10-dihydro-3-methyl-1.α.,9.α.,10.ß.-trihydroxy-,	065831-43-8	3.0E+00	2.6E-06	6.3E-10	4.2E-07	7.9E-09	3.7E-07	4.4E-06	7.1E-07
cholanthrene-2-one,3-methyl-	003343-08-6	1.5E+00	1.3E-06	3.2E-10	2.1E-07	4.0E-09	1.8E-07	2.2E-06	3.5E-07
chrysene	000218-01-9	2.6E-02	2.3E-08	5.5E-12	3.7E-09	6.9E-11	3.2E-09	3.8E-08	6.2E-09
chrysene,1,2-dihydro-,	018930-98-8	2.5E-02	2.2E-08	5.3E-12	3.5E-09	6.6E-11	3.1E-09	3.7E-08	5.9E-09
chrysene,1.α.,2.ß.-dihydroxy-1,2-dihydro-,(+)	080433-95-0	8.8E-03	7.7E-09	1.9E-12	1.2E-09	2.3E-11	1.1E-09	1.3E-08	2.1E-09
chrysene,1.α.,2.ß.-dihydroxy-3.α.,4.α.-epoxy-1,2,3,4-tetrahydro-,(+)	080433-80-3	8.2E-03	7.2E-09	1.7E-12	1.2E-09	2.2E-11	1.0E-09	1.2E-08	1.9E-09
chrysene,1.α.,2.ß.-dihydroxy-3.α.,4.α.-epoxy-1,2,3,4-tetrahydro-,(+/-)	072074-66-9	1.9E-02	1.7E-08	4.0E-12	2.7E-09	5.0E-11	2.3E-09	2.8E-08	4.5E-09
chrysene,1.α.,2.ß.-dihydroxy-3.ß.,4.ß.-epoxy-1,2,3,4-tetrahydro-,(+/-)	072074-67-0	3.7E-02	3.3E-08	7.8E-12	5.2E-09	9.8E-11	4.6E-09	5.5E-08	8.8E-09
chrysene,1.α.,2.ß.-dihydroxy-3.ß.,4.ß.-epoxy-1,2,3,4-tetrahydro-,(-)	080433-82-5	8.8E-03	7.7E-09	1.9E-12	1.2E-09	2.3E-11	1.1E-09	1.3E-08	2.1E-09
chrysene,1.ß.,2.α.-dihydroxy-1,2-dihydro-,(-)	077123-18-3	1.1E-01	9.7E-08	2.3E-11	1.5E-08	2.9E-10	1.4E-08	1.6E-07	2.6E-08
chrysene,1.ß.,2.α.-dihydroxy-3.α.,4.α.-epoxy-1,2,3,4-tetrahydro-,(+)	077123-23-0	3.5E-02	3.1E-08	7.4E-12	4.9E-09	9.2E-11	4.3E-09	5.2E-08	8.3E-09
chrysene,1.ß.,2.α.-dihydroxy-3.ß.,4.ß.-epoxy-1,2,3,4-tetrahydro-,(-)	077123-24-1	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
chrysene,3,4-dihydroxy-3,4-dihydro-,trans-,(+/-)	067175-77-3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
chrysene,3,4-epoxy-1,2,3,4-tetrahydro-,(+/-)	067694-88-6	2.9E-02	2.5E-08	6.1E-12	4.1E-09	7.6E-11	3.6E-09	4.3E-08	6.9E-09
chrysene,3.ß.,4.α.-dihydroxy-1.α.,2.α.-epoxy-1,2,3,4-tetrahydro-,(+/-)	070951-83-6	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
cycloheptapyrene,10,11-dihydro-,	110000\10\0	2.7E-02	2.4E-08	5.7E-12	3.8E-09	7.1E-11	3.3E-09	4.0E-08	6.4E-09

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
cyclopenta[cd]pyrene	027208-37-3	1.2E-02	1.1E-08	2.5E-12	1.7E-09	3.2E-11	1.5E-09	1.8E-08	2.8E-09
dibenz[a,c]anthracene,10,11-dihydroxy-10,11-dihydro-,trans-,(+/-)	072100-19-7	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
dibenz[a,h]anthracene,1,2-dihydroxy-1,2-dihydro-,trans-,(+/-)	079301-84-1	4.4E-02	3.9E-08	9.3E-12	6.2E-09	1.2E-10	5.4E-09	6.5E-08	1.0E-08
dibenz[a,h]anthracene,3,4-dihydroxy-1,2,3,4-tetrahydro-,trans-,(+/-)	093197-99-3	1.1E-01	9.7E-08	2.3E-11	1.5E-08	2.9E-10	1.4E-08	1.6E-07	2.6E-08
dibenz[a,h]anthracene,3,4-dihydroxy-3,4-dihydro-,trans-,(+/-)	074634-56-3	8.9E-01	7.8E-07	1.9E-10	1.3E-07	2.3E-09	1.1E-07	1.3E-06	2.1E-07
dibenz[a,h]anthracene,3.α.,4.ß.-dihydroxy-1.α.,2.α.-epoxy-1,2,3,4-tetrahydro-,(+/-)	131486-57-2	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
dibenz[a,h]anthracene,5,6-dihydroxy-5,6-dihydro-,trans-,(+/-)	119363-32-5	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
dibenz[a,h]anthracene,7,14-dimethyl-,	035335-07-0	2.8E+00	2.5E-06	5.9E-10	3.9E-07	7.4E-09	3.4E-07	4.1E-06	6.6E-07
dibenz[a,h]pyrene,1.ß.,2.α.-dihydroxy-3.α.,4.α.-epoxy-1,2,3,4-tetrahydro-,(+/-)	110000\07\3	1.0E+00	8.8E-07	2.1E-10	1.4E-07	2.6E-09	1.2E-07	1.5E-06	2.4E-07
dibenzo[a,h]acridine	000226-36-8	1.1E-01	9.7E-08	2.3E-11	1.5E-08	2.9E-10	1.4E-08	1.6E-07	2.6E-08
dibenzo[a,h]anthracene	000053-70-3	8.9E-01	7.8E-07	1.9E-10	1.3E-07	2.3E-09	1.1E-07	1.3E-06	2.1E-07
dibenzo[a,h]pyrene	000189-64-0	1.2E+00	1.1E-06	2.5E-10	1.7E-07	3.2E-09	1.5E-07	1.8E-06	2.8E-07
dibenzo[a,h]pyrene,1,2-dihydroxy-1,2,3,4-tetrahydro-,trans-,(+/-)	078859-21-9	1.1E+00	9.7E-07	2.3E-10	1.5E-0	2.9E-09	1.4E-07	1.6E-06	2.6E-07
dibenzo[a,h]pyrene,1,2-dihydroxy-1,2-dihydro-,trans-,(+/-)	073691-54-0	1.2E+00	1.1E-06	2.5E-10	1.7E-07	3.2E-09	1.5E-07	1.8E-06	2.8E-07
dibenzo[a,j]acridine	000224-42-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
glycol,1-(1-pyrenyl)-	073529-26-7	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
indeno[1,2,3-cd]pyrene	000193-39-5	6.7E-02	5.9E-08	1.4E-11	9.4E-09	1.8E-10	8.2E-09	9.9E-08	1.6E-08
oxirane,1-pyrenyl-	061695-74-7	3.2E-02	2.8E-08	6.7E-12	4.5E-09	8.4E-11	3.9E-09	4.7E-08	7.6E-09
oxirane,4-pyrenyl-	073529-24-5	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00

Table 3.5.7.1 Human cancer potency of PAH at different tumor sites due to different routes of exposure.....continued.

Compound	CAS #	Rel. P.	RS-lg	OR-lg	RS-st	OR-st	DR-sk	RS-lg*	RS-st*
packsene,1,2-dihydroxy-1,2-dihydro-,trans-,(+/-)	067175-76-2	6.1E-02	5.4E-08	1.3E-11	8.6E-09	1.6E-10	7.5E-09	9.0E-08	1.4E-08
phenanthrene	000085-01-8	6.4E-04	5.6E-10	1.3E-13	9.0E-11	1.7E-12	7.9E-11	9.5E-10	1.5E-10
phenanthrene,1,2-dihydro-,	056179-83-0	5.9E-04	5.2E-10	1.2E-13	8.3E-11	1.6E-12	7.3E-11	8.7E-10	1.4E-10
phenanthrene,1,2-dihydroxy-1,2-dihydro-,trans-,(+/-)	103302-39-2	7.4E-04	6.5E-10	1.6E-13	1.0E-10	2.0E-12	9.1E-11	1.1E-09	1.8E-10
phenanthrene,3,4-dihydroxy-3,4-dihydro	000569-20-0	1.0E-03	8.8E-10	2.1E-13	1.4E-10	2.6E-12	1.2E-10	1.5E-09	2.4E-10
phenanthrene,3,4-epoxy-1,2,3,4-tetrahydro-,(+/-)	066226-26-4	2.6E-03	2.3E-09	5.5E-13	3.7E-10	6.9E-12	3.2E-10	3.8E-09	6.2E-10
phenanthrene,9,10-dihydroxy-9,10-dihydro-,trans-,(+/-)	025061-61-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
pyrene	000129-00-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
pyrene,1-nitro-,	005522-43-0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
pyrene,1-vinyl-	017088-21-0	1.4E-02	1.2E-08	3.0E-12	2.0E-09	3.7E-11	1.7E-09	2.1E-08	3.3E-09
pyrene,4-vinyl-	073529-25-6	2.2E-02	1.9E-08	4.6E-12	3.1E-09	5.8E-11	2.7E-09	3.3E-08	5.2E-09
quinoline	000091-22-5	8.3E-04	7.3E-10	1.8E-13	1.2E-10	2.2E-12	1.0E-10	1.2E-09	2.0E-10
quinoline,2-methyl-,	000091-63-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
quinoline,3-methyl-,	000612-58-8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
quinoline,4-methyl-,	000491-35-0	7.4E-04	6.5E-10	1.6E-13	1.0E-10	2.0E-12	9.1E-11	1.1E-09	1.8E-10
quinoline,5-methyl-,	007661-55-4	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
quinoline,6-methyl-,	000091-62-3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
quinoline,7-methyl-,	000612-60-2	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
quinoline,8-methyl-,	000611-32-5	7.4E-04	6.5E-10	1.6E-13	1.0E-10	2.0E-12	9.1E-11	1.1E-09	1.8E-10

Table 3.5.7.1 Human potency of individual PAH is expressed as cancer risk/ng PAH/day, except the last two columns (indicated by asterisks). In the latter cases, cancer risk is expressed as risk/ng PAH/m^3. Several *CAS numbers* contain backslashes "\"; they are identification codes given to compounds for which no CAS number was found.

Abbreviations used:

DR	Dermal exposure
RS	Inhalation exposure
OR	Oral exposure
sk	risk of skin tumors
st	risk of stomach tumors
lg	risk of lung tumors

3.5.8 Comparison of Relative Potencies Estimated for Individual PAH by Different Authors

A number of authors have estimated the potency of various PAH relative to B[a]P (see table 3.5.8.1). With the exception of Rugen *et al.* (1989), the authors derived relative potencies from different species and different routes of exposure. The implicit assumption is that the relative potency of PAH is independent of the route of administration. The present report has provided evidence in support of this assumption (see section 3.4.6). Rugen had used only the mouse skin tumorigenicity experiments although different authors used different strains of mice. Collins and Alexeeff (1993) also made use of the results of genotoxic assays and structure-activity relationship to expand their list of relative PAH potencies. Willis *et al.* (1992), on the other hand, made assumptions for the potency of some of the compounds.

Although potencies have been presented in a number of reports, the estimates are not independent. For example, several reports (including Thorslund and Charnley, 1988; Krewski *et al.* 1989; Nisbet and Lagoy, 1992) derived at least some of the estimates from the same study, the study of Thorslund and Charnley (1986, 1988). Conducting risk assessment using the same data accounts for the perfect agreement between estimates from different reports for some compounds.

Our results are generally similar to the estimates derived by other authors. A wide range of values has been reported for benz[a]anthracene, ranging from a low of 0.0045 in the Rugen *et al.* 1989 study to 0.145 in the Thorslund and Charnley study (1986, 1988). Our estimate lies within the range. Most authors assigned benzo[e]pyrene a very low potency, while our estimate is 0.0. For a number of the compounds, our estimates are lower than those of the California EPA (California Environmental Protection Agency, Collins and Alexeeff, 1993). From the California EPA report, it is not clear what data were used for the estimation for a number of compounds. Thus, the reasons for the discrepancy are not clear. For some PAH, there are often no other studies with which we can compare our estimates (for example, dibenzo[a,h]acridine, dibenzo[a,h]pyrene and dibenz[a,j]acridine). Since our protocol is consistent for all compounds and our estimates compare well to the estimates of other investigators for other PAH, we expect that our estimates for these compounds accurately reflect their relative potencies as well.

Overall, we conclude that there is a good agreement between our estimates of relative potency and those reported in the literature.

Table 3.5.8.1 Relative potency of PAH estimated by different authors.

Compound	Present Study	Thorslund & Farrar, 1990	Krewski *et al.* 1989	Thorslund & Charnley, 1988	California EPA's PEF[a]	Rugen *et al.* 1989	Nisbet & Lagoy, 1992
anthanthrene	0.28	0.316	0.32	0.32	0.1		
benz[a]anthracene	0.014		0.145	0.145	0.1	0.0045	0.1
benzo[a]pyrene	1.0	1	1	1	1	1	1
benzo[b]fluoranthene	0.11	0.123	0.140	0.140	0.1	0.036	0.1
benzo[e]pyrene	0.0	0.0070	0.004*	0.004	0.01		
benzo[ghi]perylene	0.012	0.0212	0.022	0.022	0.01		0.01
benzo[j]fluoranthene	0.045	0.052	0.061	0.061	0.1	0.078	
benzo[k]fluoranthene	0.037	0.053	0.066	0.066	0.1		0.1
chrysene	0.026				0.01		0.01
cyclopenta[cd]pyrene	0.012		0.023	0.023	0.1		
dibenzo[a,h]acridine	0.11				1.0		
dibenzo[a,h]anthracene	0.89		1.11	1.11		0.60	5.0
dibenzo[a,h]pyrene	1.2				10		
dibenzo[a,j]acridine	0.0				1.0		
indeno[1,2,3-cd]pyrene	0.067	0.278	0.232	0.232	0.1	0.0060	0.1
phenanthrene	0.00064						0.001
pyrene	0.0	0.0	0.081	0.081	0.1		0.001
pyrene,1-nitro-,	0.0				0.1		

[a] - Collins and Alexeeff, 1993
* - Krewski *et al.* 1989 actually reported this value as a duplicate value for B[a]P. We assume that this value was an error and the value was meant for B[e]P.

3.6 Dose-Response Assessment Based on Whole Mixture Model (WMM)

3.6.1 Navigation Aid

Section 3.6 is a continuation of the discussion on the dose-response process in part c. Figures 3.5.1 and 3.6.1 are provided to help readers relate part c to the other parts of the document. Part c describes two dose-response assessment models for estimating the contribution of PAH to the cancer risk of mixtures. The first model is the IPM model discussed in section 3.5; the second model, the WMM, is described in section 3.6. The process is outlined in figure 3.6.1.

3.6.2 Introduction

The Whole Mixture Model (WMM) assumes that the PAH fraction[20] of all environmentally relevant PAH-containing complex mixtures are approximately equipotent when the potency is expressed in terms of the B[a]P content. The potency of the PAH fraction is assumed to be equal to the potency of a *Mixture of Standard Potency* (MSP) as defined in section 3.6.3. In order to estimate the carcinogenicity of a particular mixture, the mixture would be first analyzed for B[a]P content. The quantity determined would be multiplied by the potency of the MSP to yield the total risk due to the PAH fraction in the mixture. In essence, the model assumes that the potency of the PAH fraction is directly proportional to its B[a]P content and treats the PAH fraction as consisting of a single PAH. The advantages, disadvantages and assumptions associated with this model have been discussed in section 3.4.3.

3.6.3 Rationale

Coke oven emissions have been selected as the *Mixture of Standard Potency* for estimating the cancer risk in humans from inhalation. The rationale for the selection has been discussed in section 3.5.3 and are briefly reiterated below.

- Coke oven emissions have a very high PAH content (1.7 mg/g of organics, Albert *et al.* 1983). This phenomenon, together with the high level of occupational exposures, make PAH likely to be the major cause of lung tumors in the study subjects.
- The epidemiological studies and the risk assessment have generally been accepted in the scientific community following extensive peer review.

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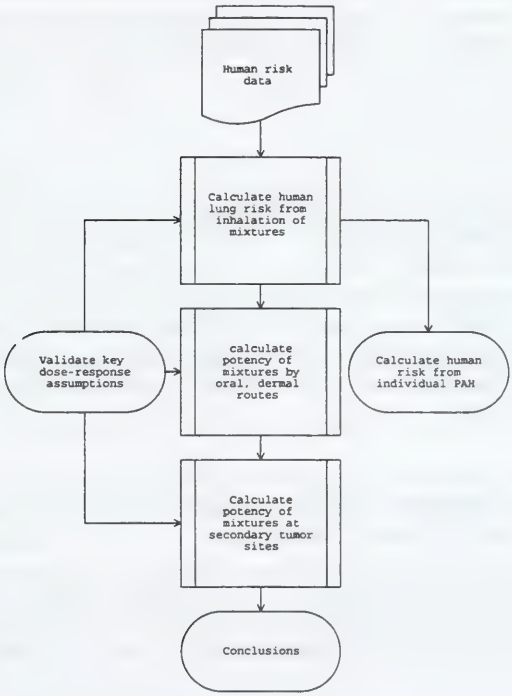
20 The PAH fraction of a mixture is defined here as the PAH that are present at levels proportional to that of B[a]P in the mixture. This fraction includes most of the commonly assayed unsubstituted PAH (see table 3.4.3.2.2.1.1.3).

Figure 3.6.1.1 Dose-response assessment using WMM - a process flow diagram


Human risk data
Calculate human lung risk from inhalation of mixtures
Validate key dose-response assumptions
calculate potency of mixtures by oral, dermal routes
Calculate human risk from individual PAH
Calculate potency of mixtures at secondary tumor sites
Conclusions

- The results are consistent with findings in other epidemiological studies and in experimental rodent studies.

Once the inhalation potency of a reference mixture in humans has been estimated, the potency of the mixture through percutaneous or oral exposure can be determined following the same rationale used for B[a]P for the same exposure routes (see section 3.5.5). In the case of B[a]P and 3-MC, exposure by inhalation is most potent and exposure by ingestion the least potent in the three rodent species (see section 3.4.7). The inhalation potency bears a specific numerical relationship to the oral and dermal potencies. We assume that the same relationships as summarized in table 3.4.9.2.3.1 also hold for mixtures.

3.6.4 Methods and Results

The derivation of the potency for the reference mixture has been described in section 3.5.3. The lifetime human lung cancer risk due to inhalation of the reference mixture was estimated to be 2.3 E-5/ng B[a]PS/m^3 (table 3.5.3.1). We previously assumed (section 3.5.4) that 0.043 mg of B[a]P would be bioavailable for absorption in the lung tissues over a lifetime exposure to 1 ng/m^3 of particulate-bound B[a]P. The same relationship was assumed for B[a]PS. Thus, lifetime exposure to 1 ng/m^3 of B[a]PS resulted in a lung tissue exposure of 0.043 mg B[a]PS. Using the risk estimate of 2.3 E-5/ng B[a]PS/m^3, the lung cancer risk in humans from inhalation of 1 ng of B[a]PS over a lifetime became 2.3 E-5/4.3 E4 or 5.4 E-10. This value could be translated into 1.4 E-5/ng B[a]PS/day (see table 3.6.4.1).

Table 3.6.4.1 Human lifetime cancer risk estimates (MLEs), expressed as (risk/ng B[a]PS)/day, at different sites for different exposure routes. B[a]PS in the present context represents the particulate-free PAH fraction of the mixture.

Route	Site of Tumor		
	Stomach	Lung	Skin
Topical			2.0 E-6
Respiratory	2.2 E-6 or 3.7 E-6 (/ng/m^3)	1.4 E-5 or 2.3 E-5 (/ng/m^3)	
Oral	4.2 E-8	3.4 E-9	

The derivation of cancer risk for other sites via different routes of exposure for the reference mixture followed the same process as for B[a]P (refer to section 3.5.5). We made use of the relative potency of PAH (relative to the potency of lung cancer from inhalation) at different sites for different

routes of exposure established in table 3.4.9.2.3.1 for the derivation. The B[a]PS potency in the lungs was multiplied by the relative potency factors to arrive at the lifetime risk estimates for other tumor sites for the various routes of exposure to PAH. The results are summarized in table 3.6.4.1.

3.7 Discussion of Dose Response Estimates

Risk estimates derived from the dose response assessment process are associated with considerable uncertainty. The presence of uncertainty applies to our process as well as those of others. Our next step, therefore, examines whether our estimates of risk are compatible with other available information. Section 3.7 discusses the *reality checks* we undertook. The comparisons we made are briefly outlined below.

- compare our assessment of risk with the assessments of other investigators (section 3.7.2)
- compare the lung cancer risk from exposure to PAH in the ambient environment to the lung cancer risk **not** attributable to other known specific causes, such as smoking (section 3.7.3)
- compare the assessments derived from human data with assessments based on extrapolation from animal data (section 3.7.4)
- compare the risk estimates obtained using the two different risk assessment models (see section 3.7.4).

The various comparisons are described in sections 3.7.2 to 3.7.4. Our conclusions are outlined in section 3.7.5.

3.7.1 Mixture of Standard Composition (MSC) and Mixture of Standard Potency (MSP)

MSC defines the PAH profile relative to B[a]P for a selected number of PAH in mixtures. We have established the PAH profile for MSC to be the average profile among a large number of mixtures from different sources (see section 3.4.3.2.2.1 and appendix C). It is possible to estimate the risk of the MSC by adding the risk attributable to individual PAH component of the mixture. The inhalation potency of the mixture, for example, is obtained by multiplying the risk/ng PAH/m^3 by the content (relative to B[a]P) of individual PAH, and by summing the products (see section 3.7.4.3, table 3.7.4.3.1).

The MSP on the other hand is meant to represent the potency of the PAH fraction of a typical mixture. It has been set to be equal to the MLE estimate of the lifetime lung cancer risk of coke oven emissions per unit exposure, as estimated from the epidemiological studies conducted by USEPA (1984, refer to section 3.7.2.2 and section 3.7.4.3 where the potencies of the two mixtures are compared).

3.7.2 Comparison of B[a]P (B[a]PS) Risk Estimates with Estimates from Other Sources

3.7.2.1 Inhalation Exposure to B[a]P

Section 3.7.2.1 compares our inhalation risk estimate with the estimates obtained by other authors using different data sets and different assumptions in their assessments. The section also explores the possible reasons for the observed differences.

It is apparent that our risk estimate for inhalation exposure to B[a]P is higher than those of other agencies and authors (see table 3.7.2.1.1). Many jurisdictions have utilized two-stage or multistage statistical models to establish the initial slope of the dose-response curve. Our *best estimate* represents our best judgment, and is based on a combination of judgment and statistical assessment and is best compared to the estimates based on the *maximum likelihood estimates* (MLEs). The MLE corresponds to the initial slope (q_1) of the dose-response curve fitted to the data with the linearized multistage model. It is the slope of the function that best fits the data and, in a statistical sense, represents the best estimate for the initial slope of the dose-response curve (USEPA, 1988). We compared our estimates with the MLEs from other studies whenever possible (see table 3.7.2.1.1). When MLEs were not available, we compared our results with those derived from the *95% upper confidence limit* (UCL) on q_1 (also called q_1^* when the UCL and linearized multistage model are used). As expected, the estimates based on UCLs are over one order of magnitude higher than the estimates based on MLEs (compare tables 3.7.2.1.1a and 3.7.2.1.1b).

Our B[a]P potency estimate is one to two orders of magnitude higher than the assessments by other authors based on MLEs and comparable to the assessments based on UCLs. In many ways, our approach, the data we used and the assumptions we made are different from most other authors. In contrast, other authors have used rather similar assumptions and data in their assessments. Not surprisingly, their estimates for the inhalation risk of PAH are relatively close to each other, while our estimate is higher.

We compared our approach to the approach used by most investigators. Most authors estimated the B[a]P potency from the study of Thyssen *et al.* (1981). In Thyssen's study, hamsters were exposed (nose only) to 0 ng/m^3, 2.2 E6 ng/m^3, 9.5 E6 ng/m^3, and 4.7 E7 ng/m^3 of B[a]P adsorbed on sodium chloride particulate. The particle diameter was between 0.2 and 0.5 μm. Animals were exposed for 4.5 hours a day for the first 10 weeks of the study, and then 3 hours a day until they died. The hamsters survived, on average, for about 96 weeks, although the animals in the highest dose group lived only for about 60 weeks. Respiratory tumors and digestive

tract tumors were observed in the two highest doses. As is often the case in experimental studies, the doses at which the effect was demonstrable were enormous and it is hard to relate such high experimental doses in absolute terms to environmental levels humans are generally exposed to.

Table 3.7.2.1.1a Comparison of our B[a]P inhalation risk estimate with the estimates of other authors - maximum likelihood estimates (MLE).

Lifetime Risk (/ng/m^3 B[a]P)	**Derivation Method**	**Reference**
1.5 E-6	Individual PAH model from the present report for B[a]P. Best estimate.	present report
7.0 E-9	Based on body weight extrapolation using the data of Thyssen *et al.* (1981) on inhalation of B[a]P in hamsters. Two stage model.	Clement Assoc., 1990
3.6 E-8	Based on (body weight)$^{3/4}$ extrapolation (Basal energy metabolism equivalence) using the data of Thyssen *et al.* (1981) on inhalation of B[a]P in hamsters. Two stage model.	Clement Assoc., 1990
6.2 E-8	Based on surface area extrapolation (surface area estimated as (body weight)$^{2/3}$) using the data of Thyssen *et al.* (1981) on inhalation of B[a]P in hamsters. Two stage model.	Clement Assoc., 1990
3.9 E-8	Based on the assumption of equal risk to hamsters and humans at equal airborne B[a]P levels. Data of Thyssen *et al.* (1981) on inhalation of B[a]P in hamsters were used as the starting point. Two stage model.	Clement Assoc, 1990
1.3 E-7	Based on the assumption of equal risk to hamsters and humans at equal B[a]P air levels. Data of Thyssen *et al.* (1981) on inhalation of B[a]P in hamsters were used as the starting point. Two stage model.	Thorslund *et al.* 1986

Table 3.7.2.1.1b Comparison of our B[a]P inhalation risk estimates with the estimates of other authors - upper confidence limits (UCLs).

Lifetime Risk (/ng/m^3 B[a]P)	Derivation Method	Reference
2.4 E-5	Based on the present report (see table 3.7.2.1.2). UCL estimate	present report
1.1 E-6	Based on surface area extrapolation (surface area estimated as (body weight)$^{2/3}$) using data of Thyssen *et al.* (1981) on inhalation of B[a]P in hamsters. Low dose extrapolation by the linearized multistage model.	Collins & Alexeeff, 1993
4.7 E-6	Based on body weight extrapolation from hamsters to humans. Data from Saffiotti *et al.* (1972) intratracheal instillation of B[a]P in hamsters were used as the starting point. Low dose extrapolation by the linearized multistage model.	Collins *et al.* 1991
4.4 E-6	Based on body weight extrapolation from hamsters to humans. Data from Feron *et al.* (1973) on intratracheal instillation of B[a]P in hamsters were used as the starting point. Low dose extrapolation by the Linearised Multistage Model.	Collins *et al.* 1991
3.7 E-7 to 1.7 E-6	Based on body weight extrapolation from hamsters to humans. Thyssen's data (1981) on inhalation of B[a]P in hamsters were used as the starting point. Risk range calculated for a range of hamster inhalation rates (0.037 m^3/day to 0.158 m^3/day). Low dose extrapolation by the linearized multistage model.	Collins *et al.* 1991
1.8 E-6	Based on the assumption of equal risk to hamsters and humans at equal B[a]P air levels. Thyssen's data (1981) on inhalation of B[a]P in hamsters were used as the starting point. Low dose extrapolation by the linearized multistage model. Upper confidence limit. USEPA has withdrawn this value from the IRIS database.	USEPA, 1984c

The Thyssen's study is favoured because the study is perceived as the most appropriate model for human exposures. There are some important differences between the human situation and the animal model, however, because the distribution of particles in the human and hamster respiratory tract is different for a given particle size (see Schlesinger, 1988). In hamsters, a larger proportion of the particles in the submicron range is deposited in the upper respiratory tract than

in humans. In humans, particles of similar size are mostly deposited in the lungs. This phenomenon may explain why Thyssen *et al.* (1981) did not observe tumors in the lungs, although tumors were clearly present in the upper respiratory tract. There are also likely species difference in the rate of transport from the deposition site (see Schlesinger, 1988 for review). Finally, sodium chloride is quite different from the typical particulate matter with which PAH are usually associated in real life (see exposure assessment document). PAH tend to adsorb onto carbonaceous particles rich with organic matter. The desorption from this particulate is slow. In those parts of the respiratory tract where mucociliary transport is efficient, PAH are likely to be transported to the GI tract before most of the PAH have an opportunity to desorb from the particulate matter (see section 3.5.4.2 and exposure assessment document). In contrast, salt dissolves readily at the deposition site, making PAH available for absorption at the site of deposition. In addition, the large amount of salt deposited onto the respiratory and digestive tract is likely to induce tissue irritation, which may affect the likelihood of tumor development.

Although most authors have used the data of Thyssen *et al.* (1981) as the starting point, some fitted the linearized multistage model to the data (see Anderson, 1983), while others selected a variation of a two stage model based on the work of Moolgavkar and Knudson (1981) and Moolgavkar and Venzon (1979). It appears that the results obtained with the two models are fairly similar (see table 3.7.2.1.1).

The final stage of estimating unit risk for B[a]P inhalation involves extrapolation from the hamster data to humans. The extrapolation is sometimes based on the assumption that the risk to hamsters and humans are equal when the B[a]P air levels are equal. This model assumes that the rates and sites of deposition are the same in the two species that are compared (Clement Associates, 1990). There is evidence, however, that this assumption does not hold (see Schlesinger, 1988 for review). The alternative approach is to extrapolate assuming a similar risk per kg body weight or per unit surface area in the two species. We have presented some evidence that this assumption may not hold for PAH (see section 3.4.4). Furthermore, the human epidemiological data for PAH-rich mixtures can in principle serve as a benchmark for evaluating the extrapolation techniques used in the determination of B[a]P potency. Assuming that all PAH-rich mixtures have similar potencies, as supported by our findings (see section 3.4.3.2.2.2, WHO, 1986 and Slooff *et al.* 1989), the extrapolated potencies of mixtures from animal data should be comparable to the potencies of mixtures derived from epidemiological studies. In reality, the extrapolation techniques that Thorslund and Farrar (1990) have applied to the mixture animal data considerably underestimate the human risk as calculated from the epidemiological data (see section 3.7.2.2). Since most investigators have used similar techniques to estimate the potency of B[a]P in humans from animal data, their B[a]P potency values are likely to have been underestimates. In addition, the use of similar risk assessment procedures and the same data sets explain why the estimates of most other investigators are similar (see table 3.7.2.1.1).

In contrast, our approach starts with a risk estimate obtained directly from humans exposed to high airborne levels of a PAH-rich complex mixture (USEPA, 1984). The potency of B[a]P is then obtained by extrapolation from the potency of the complex mixture using the relative potency model (see section 3.4.6 for details). Therefore, our estimate for B[a]P cancer potency in humans are accordingly higher than those of most other investigators.

The same relative potency approach has been used in all risk assessments by other authors to estimate the potency of PAH other than B[a]P. This implies that all the authors believe that the relative potency of individual PAH is the same in experimental animals and in humans. Furthermore, we have presented evidence in support of this assumption in section 3.4.6.2. In order that the relative potency model can be used in our dose response assessment, two conditions (described as follows) have to be satisfied.

- The relative potency of PAH is the same, regardless of the route of administration.
- The relative potency of PAH-rich complex mixtures and individual PAH is the same in humans and experimental animals.

There are ample evidence to suggest that the conditions have been met (see sections 3.4.6.2 and 3.4.6.4).

3.7.2.2 Inhalation Exposure to B[a]PS

There are a number of data sets which could have been used for the estimation of the potency of the MSP (see table 3.7.2.2.1 and Slooff *et al.* 1989 for review of the data and assessments). Some of the assessments involve ambient mixtures, where PAH contribute little to the risk of the whole mixture, so these studies are not considered here (see Nisbet *et al.* 1983 for review). Diesel emissions or cigarette smoke are also not reviewed, because PAH are not the only contributors to the toxicity of the mixtures (WHO, 1987). We therefore concentrate on the risk associated with the combustion of the coal-derived products in which the PAH content is high (WHO, 1987).

The risk estimates obtained from various assessments are compared in table 3.7.2.2.1. Most of the Maximum Likelihood Estimates (MLEs) of human lifetime inhalation risk based on human data lie within the range from 2.3 E-5 to 1.5 E-3 (see table 3.7.2.2.1a). The two low estimates (Pike *et al.* 1975 and Wilson *et al.* 1980) have been considered underestimates of the actual risk (Nisbet *et al.* 1983; see table 3.7.2.2.1c). Tuomisto and Jantunen (1987), and Lewtas *et al.* (1996) both based their assessments on the same general data set, although differences in their data selection criteria and/or risk assessment methodologies resulted in a relatively large difference, about 23-fold, between their estimates. Methodological differences in risk assessment may account for much of the apparent variations in the potencies in table 3.7.2.2.1a.

Table 3.7.2.2.1a Unit risk for B[a]PS - inhalation exposure - acceptable MLE estimates.

Lifetime Risk (/ng/m^3 B[a]PS)	Derivation Method	Reference
2.3 E-5	Coke oven workers. Linearized multistage model, based on MLE (3.9 E-5/μg benzene soluble fraction/m^3). First, we estimated MLE using USEPA (1984) data and an approach analogous to the EPA's approach for estimating UCL. Next, we converted the USEPA (1984) estimate expressed as risk per unit mass of benzene soluble fraction to risk per unit mass of B[a]PS content. 1.7 ng B[a]P/ μg benzene soluble fraction was assumed (from Albert *et al.* 1983). We adopted the result as our MLE estimate of risk for the *Whole Mixture model*.	present report; converted from USEPA, 1984
1.5 E-3	Women exposed to *smoky coal* smoke. The potency was determined as an average age-specific risk assuming continuous lifetime exposure. MLE estimate.	Lewtas *et al.* 1996
6.6 E-5	Mortality of exposed individuals to the smoke from burning *smoky coal* (Based on standardized annual death rate). The slope of the dose response curve was determined by linear regression.	Tuomisto & Jantunen, 1987
3.2 E-5	Roofers and waterproofers lung cancer mortality. Data from Hammond *et al.* (1976). Based on the linearized multistage model, MLE. Assessment conducted by Thorslund and reported by Albert *et al.* (1983).	Albert *et al.* 1983
4.2 E-4	Gas workers - lifetime risk of lung cancer mortality. Dose response by linear interpolation to zero dose risk. Based on standardized annual death rate.	Pike, 1983
1.0 E-4	Based on interpretation of existing assessments (Pike, 1983; WHO, 1986 and Tuomisto & Jantunen, 1987)	Slooff *et al.* 1989

Table 3.7.2.2.1b Unit risk for B[a]PS - inhalation exposure - acceptable UCL estimates.

Lifetime Risk (/ng/m^3 B[a]PS)	Derivation Method	Reference
3.6 E-4	Coke oven workers. Linearized multistage model, based on **UCL** (6.2 E-4/µg benzene soluble fraction/m^3). We converted the expression of USEPA (1984) estimate from risk per unit mass of benzene soluble fraction to risk per unit mass of B[a]PS content. 1.7 ng B[a]P/ µg benzene soluble fraction was assumed (from Albert *et al.* 1983). We adopted the resulting value as our UCL estimate of risk for the *Whole Mixture model.*	present report; converted from USEPA, 1984
8.7 E-5	Coke oven workers. Lung cancer mortality. Linearized multi-stage model, based on UCL. Authors converted the USEPA (1984) estimate from an expression of risk per unit mass of benzene soluble fraction to an expression of risk in terms of B[a]P content. 0.71% B[a]P content in benzene soluble extract was assumed.	WHO, 1986

Table 3.7.2.2.1c Unit risk for B[a]PS - inhalation exposure - underestimates according to Nisbet *et al.* (1983).

Lifetime Risk (/ng/m^3 B[a]PS)	Derivation Method	Reference
4.0 E-6	Gas workers excess lung cancer mortality. Dose response by linear interpolation to zero dose risk. No adjustment for less than lifetime exposure and less than lifetime observation. As a result, the risk estimate is probably **underestimated** by 12- to 14- fold (see Nisbet *et al.* 1983).	Pike *et al.* 1975
2.0 E-6	Roofers and waterproofers lung cancer mortality. Risk was judged to be **an underestimate** because the control group was from the same union as the exposed workers. Such groups were shown in the past to have an elevated cancer risk as well (Nisbet *et al.* 1983)	Wilson *et al.* 1980

Table 3.7.2.2.1d Unit risk for B[a]PS - inhalation exposure - extrapolated from animal data.

Lifetime Risk (/ng/m^3 B[a]PS)	Derivation Method	Reference
3.0 E-6	Based on the **rat lung implantation** of flue gas condensate from coal-fired furnace. Risk calculated using two stage model. Maximum likelihood estimate. We converted the author's estimate from risk expressed in terms of organic content into risk based on B[a]PS content. (0.7 ng of B[a]P/μg of condensate).	Thorslund & Farrar, 1990
1.2 E-6	Based on the **rat lung implantation** of diesel engine exhaust. Risk calculated using two stage model. Maximum likelihood estimate. We converted the author's estimate from risk expressed in terms of organic content into unit risk based on B[a]PS content. (0.092 ng of B[a]P/μg of condensate).	Thorslund & Farrar, 1990
2.1 E-6	Based on the **rat lung implantation** of gasoline engine exhaust. Risk calculated using two stage model. Maximum likelihood estimate. We converted the author's estimate from an expression of risk in terms of organic content to B[a]PS content. (0.092 ng of B[a]P/μg of condensate).	Thorslund & Farrar, 1990

The upper confidence limit estimates (see table 3.7.2.2.1b) based on human data are in the 1 E-4 range and, as expected, are for the most part about an order of magnitude higher than the MLE estimates. In contrast, the MLE potency estimates based on extrapolation from animal data are around 1 E-6, or about one to two orders of magnitude lower than the estimates based on human data (see table 3.7.2.2.1d). While the difference between the estimates based on human data and those based on experimental data may be due to differences in the mixtures themselves, the hypothesis is rather unlikely for the following reasons.

- Both the estimates based on human and animal data include mixtures derived from the pyrolysis of coal.
- There is evidence indicating that mixtures, where PAH are likely to account for much of the toxicity, have similar potencies when potency is expressed in terms of B[a]PS content (see section 3.4.3.2.2.2). This view has been presented also by WHO in 1986.

- Mixtures where PAH are not the only major contributors of tumorigenicity are not well described by B[a]PS content. Under these situations, B[a]PS is only a good indicator of the contribution of PAH and provides no indication of the risk due to other components of the mixture (WHO, 1986).

We consider the discrepancy between the estimates based on human and experimental data for complex mixtures and individual PAH very important. In estimating the potencies of PAH-rich mixtures in humans from the rat data, Thorslund and Farrar (1990) have used extrapolation techniques similar to those used for extrapolating human tumor response to B[a]P from animal data. Assuming that all PAH-rich mixtures have similar potencies, as supported by our findings (see section 3.4.3.2.2.2, WHO, 1986 and Slooff *et al.* 1989), the extrapolation techniques have underestimated considerably the human risk due to PAH-rich mixtures as calculated from epidemiological data. As human epidemiological data for PAH-rich mixtures can likely serve as a benchmark for evaluating the extrapolation techniques used in determining B[a]P potency, the B[a]P potency derived from animal data using similar techniques would have also been underestimated.

We have chosen the coke-oven emissions considered in the USEPA (1984) study as our *Mixture of Standard Potency* (MSP) for estimating the potency of PAH-rich mixtures for reasons previously discussed (section 3.5.3).

- Coke oven emissions have a very high PAH content (1.7 mg/g of organics, Albert *et al.* 1983). This phenomenon, together with the high level of occupational exposures, make PAH likely to be the major cause of lung tumors in the study subjects.
- The epidemiological studies and the risk assessment have generally been accepted in the scientific community following extensive peer review.
- The results are consistent with findings in other epidemiological studies and in experimental rodent studies.

Subsequent assessments for the PAH fraction in complex mixtures can be based on the lung cancer risk from smoky coal exposure. The smoky coal study has good exposure data and the smoking history of the subjects is not an issue (the women in the study generally do not smoke). In addition, the toxicity of the smoky coal mixture has also been investigated in rodents. However, we were unable to base our assessment on the smoky coal studies for numerous reasons. We could not obtain a copy of the study by Tuomisto and Jantunen (1987). The other smoky coal study, by Lewtas *et al.* (1996), has not undergone peer review at the time the present assessment was conducted. Furthermore, the two studies appear to differ considerably in their estimated risk.

The following are some critical issues.

- **How much are we overestimating the risk attributable to PAH?**

  We estimated the potency of the PAH fraction of mixtures by selecting mixtures with very high PAH contents and assuming that all the inhalation carcinogenicity was due to the PAH in the mixture. Most complex mixtures contain other compounds, besides PAH, that could contribute to lung tumorigenicity. However, for several mixtures containing PAH with four or more rings, the PAH fraction has been shown to account for the majority of the toxicity of the mixture as a whole (see Thorslund and Farrar, 1990).

  When the potency of a mixture is estimated by summing the risks attributable to individual PAH (the IPM), the result is about 2% to 4% of the estimated potency of the mixture as a whole (see sections 3.7.4). The IPM estimate takes into account only a few speciated PAH and thus represents the lower bound on the potency of the PAH fraction of the mixture. Taking into account a larger number of PAH in the mixture would definitely raise the risk estimate.

  Based on the work of Grimmer and coworkers (1983, 1984, 1985, 1987), as well as Thorslund and Farrar (1990), we estimate that the contribution of PAH to the cancer risk of smoky coal mixtures has been overestimated by about 0%-40%, and most likely 10%. We have made no correction for the overestimation, since we expect the overestimation to be small.

- **Is the carcinogenicity of the PAH fraction of mixtures always similar?**

  We have examined this issue elsewhere in the document (see section 3.4.3.2.2.2) and have concluded that the potency of PAH fraction (expressed in terms of B[a]PS content) is similar for all mixtures. This is based on the evidence that mixtures from widely different sources have similar PAH profiles (relative to B[a]P) and that PAH-rich mixtures tested in the same assay have approximately the same potency. This observation applies to both human and animal data.

3.7.2.3 Risk Estimates for Oral Exposure

Most investigators have estimated the risk from ingested B[a]P to be between 4.1 E-8 and 1.7 E-7 per ng B[a]P per day (see table 3.7.2.3.1). In contrast, our estimate is one to two orders of magnitude lower, at 2.6 E-9 per ng B[a]P per day. It should be noted that most studies have reported the upper bound of the estimated risk, while we have provided our best estimate. When our UCL value is compared to values obtained by other authors, the difference is substantially less (see table 3.7.2.3.1).

Table 3.7.2.3.1 Lifetime cancer potency of B[a]P in humans exposed orally.

Lifetime Risk (/ng B[a]P/day)	Derivation Method	Reference
2.6 E-9	Best estimate (MLE). UCL potency would be about 4.2 E-8.	present report
1.7 E-7	Based on Neal and Rigdon (1967) mouse data. Animals were fed B[a]P. High-to-low dose extrapolation using linearized multistage model. Three highest doses were discarded, because they did not fit the model. **Upper confidence limit.** Not recommended by Schoeny *et al.* (1991) because three top doses have been discarded.	USEPA, 1980
1.5 E-7	Based on Neal and Rigdon (1967) mouse study. Mice were fed B[a]P. Linearized multistage model. Corrected for less than life-time exposure, surface area extrapolation. **Upper confidence limit.**	Collins *et al.* 1991
4.1 E-8	Based on Neal and Rigdon (1967) mouse study. Mice were fed B[a]P. Two stage model. **Upper confidence limit.**	Thorslund & Farrar, 1990
5.4 E-8 to 1.7 E-7	Various estimates based on the data of Brune *et al.* (1989) on dietary exposure of B[a]P to rats.	Schoeny *et al.* 1991, with correction in Schoeny & Poirier, 1993
1.0 E-7	Recommended by USEPA. *Provisional guidance* estimate based on available estimates from Thorslund and Farrar (1990), Schoeny *et al.* (1991) and Schoeny and Poirier (1993).	Schoeny & Poirier, 1993

Most investigators have utilized the Neal and Rigdon (1967) feeding study in mice and estimated human risk by extrapolating from the mouse data. The extrapolation procedure has the advantage of being the most direct approach. However, we have shown that there are problems associated with the common methods (surface area and body weight extrapolations) currently used in extrapolating PAH potency from rodents to humans (see section 3.4.4 for details). In addition, the extrapolation is usually based on mouse data alone, and data from other species are not considered. Our analysis shows that there may be species-specific differences in sensitivity to PAH administered orally (see section 3.4.7).

Our approach differs from the approaches used by other authors in estimating the oral potency in humans and we rely on different data sets and assumptions. Our method requires the consolidation and analysis of data generated by different authors tested in different assay systems. The practice tends to reduce the quality of our data and adversely affects the reliability of our conclusions. On the other hand, we are able to use experimental data from three different rodent species rather than just the mouse data. We believe that the use of data from several rodent species increases the overall reliability of the conclusions because we are less dependent on a single species.

Our method is less direct. We estimate the oral potency from the estimated respiratory potency. Our relative potency estimate for different PAH via the respiratory and oral routes varies within an order of magnitude, depending on the species and possibly methodological differences. Therefore, our oral potency estimate for PAH is probably correct within an order of magnitude.

In our attempt to establish the relationship between the potencies of PAH for different routes of exposure (inhalation, oral and topical routes) in rodents, we encountered considerable gaps in the data base that need to be filled so as to arrive at reliable estimates. A high priority should be put on conducting studies specifically designed to fill these data gaps.

3.7.3 Fraction of Lung Cancer Risk Attributable to PAH

Section 3.7.3 examines whether the estimated risk for PAH is compatible with the lung cancer risk reported among the general Ontario population and with the ambient levels of B[a]P in the environment. This analysis is useful for two reasons.

- It provides a needed perspective on the magnitude of the contribution of PAH to the total lung cancer risk experienced by the human population relative to other known carcinogens.
- It provides some evidence that our risk estimate is within reasonable bounds. If the estimated lung cancer risk at normal environmental exposure levels based on our unit risk estimate for B[a]P exceeds the actual total lung cancer incidence, our unit risk estimate would probably be incorrect. However, if the risk that can be attributed to PAH is less than the risk due to all causes other than smoking, the finding is not a proof that our estimates are correct.

Table 3.7.3.1 puts the lung cancer risk due to PAH in perspective. The risk attributable to PAH is compared to the total lung cancer risk (all causes) and to the risk of lung cancer not attributable to smoking among the Ontario population. It is apparent, at least in 1992, that PAH account for about 2% of the lung cancer risk **not** attributable to smoking among Ontarians, which is within the bounds of the expected magnitude of risk.

We have also considered the contribution of radon in homes to lung cancer risk. The lifetime probability of developing lung cancer from radon exposure in homes in the US (based on USEPA, 1987 estimate of 5000 to 20000 cases a year and a population of 250 million) is about 2 E-5 to 8 E-5, or less than 1% of the total lung cancer risk. Assuming radon contributes the same level of lung cancer risk in Ontario (1%), the little impact radon exposure has on the general population is not expected to overshadow the effect of PAH. Radon is therefore not included in further discussion.

Table 3.7.3.1 Lung cancer risk due to PAH exposure versus smoking.

Lung Cancer Risk Type	Risk to Ontarians	% Lung Cancer Risk	% Lung Cancer Risk Not from 1st Hand Smoking
Total risk	6.7 E-2[a]	100	-
Risk not from smoking	1.9 E-2[b]	28	100
Risk from PAH	3.6 E-4	0.54	1.9
Risk due to PAH produced by a smoker in residence	8.6 E-5	0.13	0.45

[a] - Statistics Canada *et al.* 1992
[b] - IARC, 1986

3.7.4 Comparison of Risk Estimates Based on WMM and IPM

Section 3.7.4 compares the risk estimates determined using the two different risk assessment models described in the present report. We have indicated previously (section 3.4.3.3) that the results based on the IPM likely underestimate the risk, while the WMM may somewhat overestimate the risk. This section examines our hypothesis and determines the actual difference between the IPM and WMM estimates. We use the data for *smoky coal* emissions (see section 3.7.4.1), *coke oven* emissions (see section 3.7.4.2), as well as the MSC and MSP (see section 3.7.4.3) for the analysis.

3.7.4.1 Smoky Coal Emissions

In order to calculate the risk attributable to PAH in smoky coal emissions using the IPM, we determined the levels of each PAH in the mixture relative to B[a]P (column 3 in table 3.7.4.1.1). Next, we multiplied the relative content of each PAH by its relative potency (column 2 of table 3.7.4.1.1). The product which represents the risk due to individual PAH present in the

mixture per ng B[a]P per m^3 is presented in the rightmost column of table 3.7.4.1.1. We estimated the human lung cancer risk attributable to those PAH for which analytical data and toxicological data were available to be 1.9 E-6/ng B[a]P/m^3.

Table 3.7.4.1.1 Lung cancer risk attributable to some PAH in smoky coal emissions - calculated by IPM.

Compound	Risk (/ng/m^3)	Relative Content[a]	$Risk_{PAH}$ (/ng Ba]P/m^3)
Benz[a]anthracene	2.1 E-8	1.7	3.6 E-8
Benzo[a]pyrene	1.5 E-6	1.0	1.5 E-6
Indeno[1,2,3-cd]pyrene	9.9 E-8	0.62	6.1 E-8
Benzofluoranthenes	1.6 E-7[b]	1.6	2.6 E-7
Dibenz[a,j]acridine	0.0	0.037	0.0
Total			1.9 E-6

[a]Mumford *et al.* 1990.
[b]the b isomer.

Table 3.7.4.1.2 Comparison of risk estimates for smoky coal smoke: derivation from epidemiological data versus summation of risk attributable to some PAH found in smoky coal emissions.

Lifetime Risk (/ng/m^3 B[a]PS)	Derivation Method	Reference
6.6 E-5	Mortality from exposure to *smoky coal* (based on standardized annual death rate). The initial slope of the dose response curve was determined by linear regression.	Tuomisto & Jantunen, 1987
1.5 E-3	Women exposed to *smoky coal* smoke. The slope of the dose response curve was determined as an average age-specific risk assuming continuous lifetime exposure. MLE estimate.	Lewtas *et al.* 1996
1.9 E-6	Estimated by adding risks attributable to individual PAH	present report

Table 3.7.4.1.2 compares the IPM risk estimate to the estimate derived from epidemiological data. As expected, the approach based on summing the risk of individual PAH is an underestimate of the epidemiologically derived risk. In this example, the IPM estimate of risk attributable to individual PAH is about 8.3% (1.9 E-6/2.3 E-5 - see table 3.7.4.1.2) of the Tuomisto and Jantunen (1987) estimate which was based on human exposure to smoky coal emissions. The difference is even more pronounced when the IPM estimate is compared to the assessment of Lewtas *et al.* (1996).

3.7.4.2 Coke Oven Emissions

Section 3.7.4.2 compares the risk estimates for coke oven emissions based on human epidemiological data against summing the risks attributable to some of the PAH found in the mixture. Table 3.7.4.2.1 provides the risk estimation for the PAH fraction of coke oven emissions using the IPM model. The process is analogous to the one described in section 3.7.4.1.

Table 3.7.4.2.1 Lung cancer risk attributable to some PAH in coke oven emissions - calculated using IPM.

Compound	Risk (/ng/m^3)	Relative Content[a]	Risk$_{PAH}$ (/ng B[a]P/m^3)
anthanthrene	4.1 E-7	0.14	5.7 E-8
benz[a]anthracene	2.1 E-8	1.9	4.0 E-8
benzo[a]pyrene	1.5 E-6	1.0	1.5 E-6
benzo[b+j+k]fluoranthene	9.4 E-8*	0.53	5.0 E-8
benzo[c]phenanthrene	3.4 E-8	0.49	1.7 E-8
benzo[e]pyrene	0.0	0.46	0.0
benzo[ghi]perylene	1.8 E-8	0.52	9.4 E-9
chrysene/triphenylene	3.8 E-8**	2.2**	8.4 E-8
phenanthrene	9.5 E-10	9.5	9.0 E-9
pyrene	0.0	3.6	0.0
Total			1.8 E-6

* - Average of the b, j and k isomers.
** - Risk and relative content of chrysene.
[a] - Bjørseth and Becher, 1986.

Table 3.7.4.2.2 Comparison of risk estimates for coke oven emissions: derivation from epidemiological data versus summation of risk attributable to some PAH found in the emissions.

Lifetime Risk (/ng/m^3 B[a]PS)	Condition	Reference
2.3 E-5	Coke oven workers, lung cancer mortality, linearized multistage model, based on MLE (3.9 E-5 /μg benzene soluble fraction/m^3). We converted USEPA (1984) risk estimate expressed in terms of the mass of benzene soluble fraction to an expression of risk in terms of B[a]PS content. 1.7 ng B[a]P/ μg benzene soluble fraction was assumed (from Albert *et al.* 1983).	present report, converted from USEPA, 1984
1.8 E-6	Estimated by adding risks attributable to individual PAH.	present report

The comparison between the two risk assessments is shown in table 3.7.4.2.2. The risk estimate based on the IPM model is approximately 7.8% of the risk derived from epidemiological studies.

3.7.4.3 Comparing Potencies of Two Standard Mixtures, *MSC* and *MSP*

This section compares the risk estimated for the two *standard mixtures* as defined in section 3.7.1. The MSC is defined by its PAH profile, the average PAH profile as determined from a wide range of environmentally relevant, PAH-rich complex mixtures (see section 3.4.3.2.2.1). We have derived the potency of the MSC by summing the risks attributable to individual PAH in the mixture (see table 3.7.4.3.1). The potencies of individual PAH have been presented in section 3.5.7.

In contrast to MSC, which is defined by its composition, MSP is defined by its potency as established from the epidemiological studies. The potency of MSP has been set to be equal to the potency of the coke oven emissions described by USEPA (1984). The composition of MSP is not defined and is not necessarily identical to the composition of either MSC or coke oven emissions.

Table 3.7.4.3.1 Comparing potencies of MSP and MSC.

Compound	Risk (/ng/m^3)	Relative Content in MSC	Risk$_{PAH}$ (/ng B[a]P/m^3)	% Risk of MSC[#]	% Risk of MSP[##]
Benzo[a]pyrene	1.5 E-6	1.0	1.5 E-6	63	6.5
Anthanthrene	4.1 E-7	0.31	1.3 E-7	5.4	0.57
Anthracene	-	1.6	-	-	-
Benz[a]anthracene	2.1 E-8	1.2	2.5 E-8	1.0	0.11
Benzo[e]pyrene	0.0	1.1	0.0	0.0	0.0
Benzo[ghi]perylene	1.8 E-8	1.0	1.8 E-8	0.75	0.078
Benzofluoranthenes	9.4 E-8*	2.5	2.4 E-7	10	1.0
Chrysene/Triphenylene	3.8 E-8**	2.0	7.6 E-8	3.2	0.33
Coronene	-	0.34	-	-	-
Dibenz[a,h]anthracene	1.3 E-6	0.28	3.6 E-7	15	1.6
Fluoranthene	-	3.8	-	-	-
Indeno[1,2,3-cd]pyrene	9.9 E-8	0.86	8.5 E-8	3.5	0.37
Perylene	-	0.45	-	-	-
Phenanthrene***	9.5 E-10	4.3	4.1 E-9	0.17	0.018
Pyrene	0.0	2.8	0.0	0.0	0.0
Acenapthylene***	-	0.36	-	-	-
Acenapthene***	-	0.71	-	-	-
Fluorene***	-	1.6	-	-	-
Total			2.4 E-6	102%	11 %

[#] - Risk of MSC = 2.4 E-6/ng B[a]P/m^3 (table 3.7.4.3.1).

[##] - Risk of MSP = 2.3 E-5/ng B[a]P/m^3 (sections 3.5.3 and 3.6.4).

* - Average of b, j, and k isomers.

** - Risk due to chrysene.

*** - Profile applicable to particle-bound component only.

Based on the results presented in table 3.7.4.3.1, the potency of MSC has been determined to be 2.4 E-6 ng B[a]P/m^3. On the other hand, MSP has been set to 2.3 E-5 ng B[a]PS/m^3 (see section 3.6.4). Thus, the human lung cancer risk from inhaling a typical PAH-rich mixture based on the MSC concept is 10% of the risk estimate based on the MSP concept (2.4 E-6/2.3 E-5). The finding is consistent with our expectation that the estimation based on some speciated PAH present in the mixture is an underestimation of the risk relative to the risk value derived from treating the PAH fraction as a whole. The finding is also consistent with those of Thorslund and Farrar (1990), who found that the most commonly assayed PAH (which are just a few) contributed less than five percent of the toxicity of the PAH fraction in three out of four complex mixtures that were studied. Therefore, the difference in the potency of MSC and MSP can be interpreted as strong indication that the approach based on summing the risk of individual PAH significantly underestimates the potency of the PAH fraction of a mixture.

It is also apparent from table 3.7.4.3.1 that B[a]P contributes an overwhelming proportion of the activity of the MSC (about 63% of the total mixture activity). Other PAH that contribute significantly to the risk of MSC are dibenz[a,h]anthracene, anthanthrene and benzofluorathenes which together comprise an additional 30% of the estimated MSC activity.

3.7.5 Conclusions

We have estimated the human cancer risk from exposure to a mixture containing PAH using two different approaches. The first approach estimates the potency of the mixture as a whole, while the other approach estimates the potency of the PAH mixture as the sum of risks attributable to individual PAH component. We believe that the first approach may lead to a slight overestimation, while the second approach leads to an underestimation of the true risk. For the specific mixtures examined, the estimate based on speciated PAH approach is approximately 8 to 10% of the estimate based on the whole mixture approach. We do not expect the difference in the risk estimates would vary much from mixture to mixture, unless the list of PAH considered in IPM for the estimation of the potency of MSC can be expanded. Our estimate of human lung cancer risk due to inhalation of PAH is higher than the estimates by other authors and agencies who have derived their values from animal data. Our estimates are derived from risk assessments using human data, so extrapolation from experimental animals to humans is not necessary. We have indicated that the extrapolation methods used by most investigators may not be appropriate for PAH. Furthermore, our results are compatible with the assessments which were conducted by WHO (1986) and Sloof *et al.* (1989) based on human data. Based on the above considerations, we conclude that our estimate of lung cancer risk from inhaling PAH mixtures is relatively reliable.

Human cancer risk from PAH ingestion has also been evaluated. On the whole, our estimate is lower than the published values (refer to section 3.7.2.3). Our ingestion risk estimate is much lower than our inhalation risk estimate. Although PAH in the diet do contribute a measurable risk, we have concluded that risk from PAH in the diet is only a relatively small fraction of the overall risk of stomach tumors (discussed in the exposure assessment document).

Finally, we have estimated human cancer risk from dermal exposure to PAH. Our estimate of dermal unit risk approaches the inhalation unit risk. We are not aware of any other dose-response assessment that has examined the dermal route of exposure.

3.8 Modifying Factors

The cancer risk from exposure to PAH-rich mixtures calculated in sections 3.6 and 3.7 are especially relevant to the typical healthy young adult male population because most of the epidemiological studies (section 3.3.1) were conducted on this particular population who have been occupationally exposed to PAH. In contrast, it is the general population that would be impacted by environmental exposures. The general population, unlike the healthy worker population, is more heterogeneous, and includes also the very young, the very old, both the males and females, and the sick. Exposures can be long-term or short term. Epidemiological studies offer few clues about how age, sex, duration of exposure and population heterogeneity affect cancer risk resulting from PAH exposure. It appears that women who were exposed to smoky coal experienced approximately the same lung tumor risk per ng B[a]PS as males who were similarly exposed in the coke oven studies. This section will examine the possible effect of the various parameters, such as population heterogeneity, exposure duration, age and sex, on the magnitude of cancer risk from exposure to PAH in humans.

3.8.1 Heterogeneity in Human Population

No epidemiological studies have examined the difference in sensitivity in the human population to PAH-rich mixtures. Furthermore, no specific experiments have been conducted to test the distribution of sensitivity in the heterogeneous wild-type rodent population. Therefore, in order to evaluate the variability in PAH sensitivity in a genetically diverse human population, we examine the difference in PAH sensitivity in inbred strains of mice.

We choose the mouse data because mice have been most extensively studied for strain-specific sensitivity to PAH. Data on other animal species are not sufficient for this type of analysis. Since each inbred mouse strain has a different genetic makeup, each strain can be assumed to represent a "sample" from the wild population. The approximation is obviously not perfect due to two main reasons. 1. There is potential difference in the heterogeneity between different species (mouse versus

humans). 2. The selection of strains used for carcinogenicity testing is unlikely to be random. For example, the SENCAR mouse strain has been specifically bred for its sensitivity to DMBA, a PAH, while other strains are less sensitive (see table 3.8.1.1).

Table 3.8.1.1 Sensitivity of mouse strain to B[a]P carcinogenicity (complete carcinogenesis assay).

Mouse Strain	Estimated Slope (% mice/μg B[a]P)
SENCAR	1.859
C57BL/6	0.091
Swiss ICR/HA	0.110

Data are from:
SENCAR mice - Nesnow et al. 1982
C57/BL mice - Reiners et al. 1984
Swiss ICR/HA - Cavalieri et al. 1978, 1983

Slaga *et al.* (1982) has shown that C57BL/6 mice are not susceptible to the action of B[a]P in the tumor-initiation model. SENCAR mice, on the other hand, are more susceptible than the CD-1 strain. The difference in sensitivity between the strains is thought to be largely due to the relative insensitivity of C57BL/6 to TPA promotion (Slaga *et al.* 1982). In the complete carcinogenicity assay (see table 3.8.1.1), the relative sensitivity among the strains is different. C57BL/6 mice do respond to PAH, but are about an order of magnitude less sensitive than the SENCAR mice. Since the SENCAR strain has been inbred for its sensitivity to DMBA, its sensitivity likely approaches the upper bound of PAH sensitivity. The other mouse strains are less sensitive to B[a]P than SENCAR by up to 20-fold.

The inter-strain difference in sensitivity to PAH in the two assays is at least partly due to the differential sensitivity to the promoting agent (Slaga *et al.* 1982), be it PAH itself or another promotor such as TPA. In the complete carcinogenicity model, the strains examined differ in their sensitivity to PAH by about 20-fold, indicative of the range of sensitivity in the general mouse population. It is quite likely that the range would be larger in the wild rodent population.

Based on the mouse data, we assume that the variation in sensitivity to PAH in the human general population exceeds one order of magnitude.

3.8.2 Duration of Exposure

Throughout the present dose response assessment, we have assumed that human environmental exposure to PAH occurs continuously over a long duration and at a fixed level. This

assumption may not always hold in the real world, as human exposure may vary substantially during the day, from day to day, or from season to season. For example, people may breathe in clean air during the day. When they start up an old-fashioned wood stove or an open fireplace in the evening, the exposure levels can rise quickly by several orders of magnitude. The summer barbecue season may result in a sharp increase in the PAH content in people's diet. Moving from a living accommodation located in the rural area to an area near a major industrial point source may affect a person's exposure for many years. The challenge, therefore, lies in the estimation of the effect of such intermittent exposures on risk.

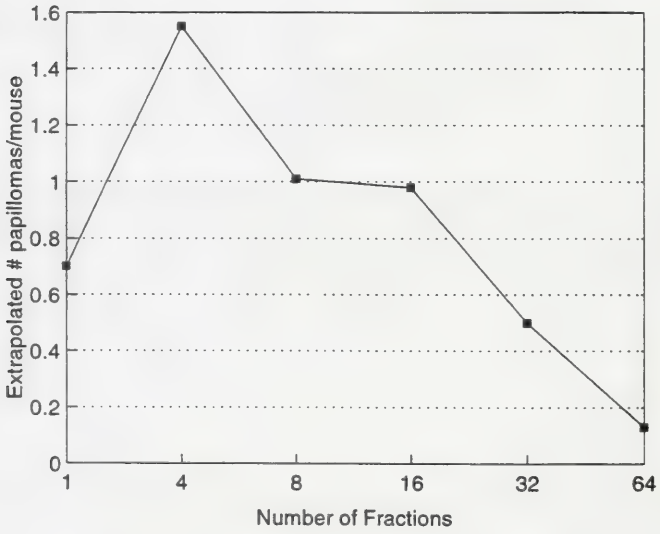


Figure 3.8.2.1 Effect of exposure schedule on tumor multiplicity in mice. Animals were administered B[a]P topically. (Modified from Burns, 1989).

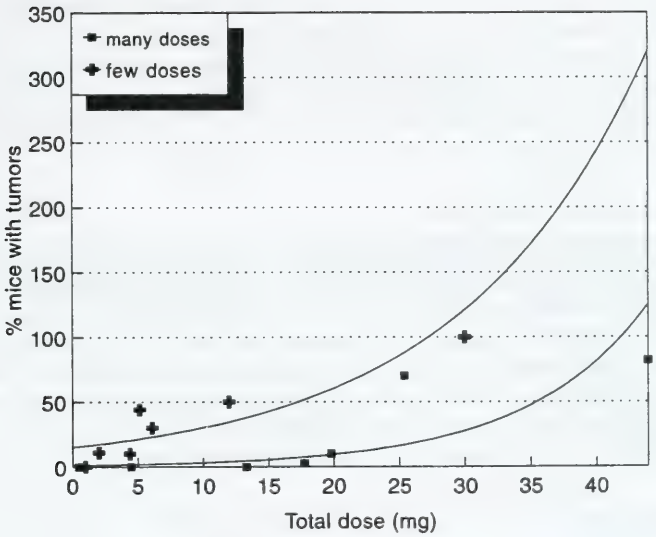


Figure 3.8.2.2 Relative sensitivity of mice to B[a]P by oral administration. Comparison of effect of many small doses versus effect of a single dose or a few large doses.

We are unable to locate data which would allow for a rigorous assessment on the effect of intermittent exposure. We therefore ask a simpler, more qualitative question. "Is there a difference in tumorigenicity as a result of exposure to a given amount of PAH, either as a single dose or as a series of doses?"

Burns Study (1989)

The work of Burns (1989) provides the most reliable answer to the question (see figure 3.8.2.1). Mice were initiated with 64 μg of B[a]P applied to the skin in 1, 4, 8, 16, 32 or 64 dose fractions. Promotion was carried out for a maximum of 350 days with 5 μg of TPA 3 times weekly. Administration of the initiator in 4 dose fractions produced the maximum tumor multiplicity. As the number of dose fractions increased, the response became smaller. For example, the response resulting from dosing the animals in 64 fractions was about 1/5th the response from the same amount of PAH delivered in one single dose, and 1/10th of the response when the same amount of PAH was administered in four dose fractions.

Neal and Rigdon Study (1967)

The Neal and Rigdon (1967) study showed a similar trend in mice. Two groups of animals were exposed to the same total dose of B[a]P in their diet. However, the number of treatments varied. One group of mice received B[a]P for 1 to 7 days and the other group for 110 days. The dose response curves (Figure 3.8.2.2) suggest that, for the same total dose, B[a]P is more effective as a single dose or when administered in a few large doses than when administered in many smaller doses over a longer period of time. Furthermore, the two dose-response curves do not appear to be parallel. The difference in the effectiveness of PAH in inducing tumor when administered in a few versus many treatments may be particularly pronounced when the total dose is low.

Other Studies

We further investigated the apparent dose rate effect observed in both the Burns, and the Neal and Rigdon studies. Since only these two studies specifically examined the dose rate effect of PAH, we compared the response of animals to a single exposure in one study to the response of animals to multiple exposures in another study. The studies might have been conducted by different investigators. A procedure similar to the one that has been previously used for species comparison as described in section 3.4.4.1.1 was adopted for the dose rate analysis. Data used in the analysis were selected from appendix A according to the following criteria.

- We paired studies that had similar experimental protocol with the exception of the dosing scheme. One study administered B[a]P or 3-MC in a single dose, and the second study administered the same compound in a number of doses or as a sustained release.
- We determined the tumor response as incidences/ng for each study and for each study pair, the ratio of the responses.

Our results indicate that a given dose is about 15 times more potent when administered in one single treatment than when administered as multiple small doses over a longer period of time (see table 3.8.2.1). Our findings are consistent with the results of the Burns study as well as the Neal and Ridgon study. This suggests that steady continuous exposure to PAH may result in a lower risk (mg for mg) than occasional (intermittent) exposure to high doses of PAH. Based on the findings, we assume that humans exposed to a large dose of PAH for a short duration (days) would be at about an order of magnitude higher risk than a human population exposed to the same total amount of PAH over a longer period of time (months).

Table 3.8.2.1 Effectiveness of tumor induction by B[a]P or 3-MC administered in a single dose versus multiple doses.

Species	Route	PAH	Tumor Site	Single Dose /Multiple Dose*	Reference
mouse	oral	3-MC	lung	330	Stoner *et al.* 1984; Lorenz & Stewart, 1948
mouse	respiratory	3-MC	lung	21	Nettesheim & Hammons, 1971
mouse	oral	B[a]P	forestomach	17	Pierce, 1961; Wattenberg *et al.* 1979
rat	oral	3-MC	breast	14	Huggins *et al.* 1959, 1961
hamster	respiratory	B[a]P	respiratory	1.1	Ketkar *et al.* 1977; Saffiotti, 1972
mouse	topical	B[a]P	skin	7.0**	Burns, 1989
Geometric Mean				15	

* - Tumor potency expressed as incidence per ng.

** - Tumor potency expressed as number of (tumors/mouse)/ng.

3.8.3 Age and Sex

Available experimental data suggest that neither the age nor the sex of the exposed rodents affect their response to PAH. The occurrence of breast tumors in rats, normally in females only, appears to be the only exception. For example, the males and females did not show marked difference in their sensitivity in the mouse skin assays (Nesnow *et al.* 1982). Furthermore, Peto *et al.* (1975) demonstrated, in a well designed experiment, that the same length of treatment with the same dosage of PAH resulted in the same tumor response in mice, regardless of the age of the animals when treatment was first initiated.

3.9 Evaluation of Uncertainty

3.9.1 Introduction

One can consider risk assessment as a process designed to reduce the uncertainty associated with the estimation of the potency of a toxicant to a tolerable level. For example, experimental results may indicate that a given compound could be toxic to some animals. At this early stage of the risk assessment process, the uncertainty in our knowledge about the risk of the toxicant to humans could be large. The risk assessment process reduces the uncertainty step by step, until at the end of the process, the uncertainty is considerably less than at the beginning.

It is useful to distinguish between two types of uncertainty, mathematical uncertainty and uncertainty due to the use of professional judgment in place of data or data analysis. The mathematical uncertainty stems from variability in the data and from the numerical processing of the data. Mathematical uncertainty has long been recognized and described in risk assessments. For example, the use of the *maximum likelihood estimate* (MLE) and the *upper confidence limit* (UCL) provides a measure of numerical uncertainty. The MLE represents the best fit to a given set of data. If low dose extrapolation uses the linearized multistage model, the MLE corresponds to the best estimate of the initial slope (q_1) of the dose-response curve. In contrast, the UCL q_1^* corresponds to the 95% upper confidence limit of the q_1 estimate. Thus, the use of UCL incorporates only the uncertainty associated with the variability in the data.

One should pay more attention to the uncertainty that results from the use of professional judgment, as it is usually the largest source of error. This type of uncertainty results from the necessity of repairing gaps in the data by complementing incomplete or unreliable data with judgment, when risk assessor has to use unvalidated assumptions, judgment and generalizations in place of analysis and interpretation of available data. Risk assessors often argue that only data which meet certain quality criteria should be used. However, following strict criteria can limit considerably the database that can be used and force the investigator to perform the assessment using poorly substantiated assumptions. **We believe that, in general, careful evaluation of existing (and even imperfect) data permits risk assessment to have a lower overall uncertainty and is more reliable than replacing data analysis with unvalidated assumptions.** In our assessment, we often depend on imperfect or incomplete sets of data to validate our assumptions. Our approach has its own uncertainty. We therefore attempt to evaluate the magnitude of uncertainty for each major step in our dose-response assessment.

In most instances, performing a numerical evaluation of the uncertainty, especially the type associated with judgment, in a risk assessment is not feasible. Instead of numerical values, we express

the uncertainty in terms of the range of values, in orders of magnitude, within which the estimate is expected to lie. The next section describes the process used in assessing the uncertainty of our risk assessment.

3.9.2 Description of Uncertainty

In section 3.9.2, we review each major step of our risk assessment and evaluate the likely contribution of each major step to the overall uncertainty of the assessment.

3.9.2.1 Human Inhalation Risk from Exposure to Representative PAH-Rich Mixtures

Both risk assessment models, the individual potency model (IPM) and the whole mixture model (WMM), start with the potency estimates of known typical PAH-rich mixtures in humans. The study (USEPA, 1984) we have selected as the basis for deriving human lung cancer potency for both individual PAH and the whole mixtures has many good features.

- Coke oven emissions have a very high PAH content (1.7 mg/g of organics, Albert *et al.* 1983). This phenomenon, together with the high level of occupational exposures, make PAH likely to be the major cause of lung tumors in the study subjects.
- The epidemiological studies and the risk assessment have generally been accepted in the scientific community following extensive peer review.
- The results are consistent with findings in other epidemiological studies and in experimental rodent studies.

Therefore, we expect that the error associated with the lung cancer risk estimate of humans from exposure to coke oven emissions is well within an order of magnitude

3.9.2.2 Extrapolation from Inhalation Risk to Oral and Dermal Risk

The steps considered in section 3.9.2.2 include the estimation of the proportion of airborne PAH in the respiratory tract that is available for absorption as well as the estimation of the relative potency of PAH applied topically, orally or into the respiratory tract.

3.9.2.2.1 Bioavailability of PAH to Lungs as a Function of Airborne Levels

The main source of uncertainty is associated with the determination of the proportion of inhaled particulate that is retained in the respiratory tract and the proportion of particle-bound PAH that are bioavailable in the respiratory tract.

The retention of particles in the respiratory tract is dependent on the particle deposition rate which in turn depends on the size of the particles. The deposition rates are quite variable. In the human lungs, for example, deposition ranges from about 10% to 60% (see Schlesinger, 1988). There is little data that allow one to estimate accurately the proportion of inhaled PAH that is transported from the respiratory tract to the GI tract and the lymphatic system, and the proportion that is eventually absorbed into the blood stream in the respiratory tract.

We expect that the assumption of 10% bioavailability for nasal breathing is correct to well within an order of magnitude, i.e. the bioavailability is expected to lie between 1% and 100%. There is a greater chance that the true bioavailability is less than 10%, rather than over 10%.

3.9.2.2.2 Relative Potency of PAH by Inhalation, Ingestion and Dermal Exposure

No study has been specifically designed to evaluate the potency of PAH for one route of exposure relative to another route. Our estimates of relative potency are therefore based on a pool of different studies by different authors. Although we try to select only studies that used similar protocols, it is likely that some or part of the route-specific differences in PAH potency are in fact due to methodological differences in the studies used for comparison. The magnitude of the uncertainty can be estimated from table 3.4.6.2.2.1. Table 3.4.6.2.2.1 presents the relative sensitivity of different species to both B[a]P and 3-MC dependent on the route of exposure. We assume that the potency of PAH relative to B[a]P is independent of species and the route of administration.

Table 3.4.7.4.2 can also be used to estimate the uncertainty in the relative potency of PAH due to different routes of exposure. Table 3.4.7.4.2 lists the tumor potencies of PAH at particular organ sites relative to tumors at the respiratory tract in different animal species. The relative sensitivity of different animal species that ingested PAH or were dermally applied PAH ranges within one order of magnitude, while all species are equally sensitive to inhaled PAH.

In conclusion, we estimate that the sensitivity to PAH due to different routes of exposure lies within one to two orders of magnitude. The overall extrapolation of the risk from inhalation of PAH to ingestion or dermal exposure is estimated to be associated with an uncertainty of less than two orders of magnitude.

3.9.2.3 Tissue-Specific Risk Assessment

We have estimated the relative sensitivity in the response to PAH at two organ sites, the stomach and the lungs, after oral and inhalation exposure. The estimates were derived mostly

from studies that considered both organ sites in the same animals. This practice alleviates the concern that the observed difference could be due to differences in experimental design. There are only a few studies that examined tumors in both the stomach and the lungs (see section 3.4.8) and the study results in general agreed well with each other. The only exception is the oral hamster study. B[a]P induced tumors only in the forestomach, but not in the trachea. In contrast, B[a]P induced tumors in both tissues in the two mouse studies (see table 3.4.9.2.1.2).

We estimate that the uncertainty in the tumor induction potency of PAH at one site relative to another site is less than an order of magnitude.

3.9.2.4 Potency of B[a]P

The potency of B[a]P by the respiratory route was estimated from the human potency of mixtures using the relative potency approach (see section 3.5.3). The evidence in support of the relative potency approach has been discussed extensively in section 3.4.6. Based on the evidence, we estimate that only a small error is associated with this extrapolation. Some of the error stems from the uncertainty associated with the estimation of the potencies of mixtures in humans and from the estimation of potencies of mixtures and B[a]P in experimental animals. Not only that there is a very good agreement between the various estimates for lung cancer potency of mixtures in humans (see section 3.3.1), the animal experiments all have high quality dose-response curves (see section 3.5.7).

We therefore conclude that the uncertainty associated with the estimation of the inhalation potency of B[a]P in humans is less than an order of magnitude.

3.9.2.5 Relative Potency of Individual PAH to B[a]P

The relative potency of various PAH (to B[a]P) was estimated by comparing the potency of a given PAH to the potency of B[a]P (see section 3.5.6). Some uncertainty may result from the standardization of the time of observation for all experiments, but we expect the error to be small. It is possible that, in some instances, non-tumorigenic compounds may have been assumed to have low tumorigenic activity if the experiment had been allowed to run for a short duration. Conversely, the carcinogenicity of some strongly carcinogenic compounds may have been somewhat underestimated if the experiments lasted a long time. We do not expect, however, that these circumstances alter the relative potency estimates significantly. Combining experiments from different laboratories is not expected to significantly add to the uncertainty, because the experiments were conducted under very similar conditions.

We have also compared the relative potency values based on potencies established using different statistical methods, for example, linear regression versus linearized multistage model versus $ED_{0.10}$ (refer to section 3.5.6.2). All three methods yield similar results (refer to section 3.5.6.2). Thus, selection of the dose-response model has little effect on the uncertainty. On the other hand, in some experiments, the lowest dose already achieved a maximum response (80% above control) in the animals. Under these circumstances, the potency of the compound could have been underestimated.

Overall, we estimate that the uncertainty associated with the evaluation of the relative potency of individual PAH (to B[a]P) would be less than an order of magnitude.

3.9.2.6 Potency of PAH-Rich Mixtures Based on IPM

Complex mixtures usually contain a very large number of PAH, but only a few of them are chemically characterized. Perhaps more significantly, the potencies of the vast majority of PAH compounds found in the environment are unknown. Furthermore, in the absence of other information, additivity of risk is assumed. As a result, the estimation of the contribution of the PAH fraction to human cancer risk from exposure to the whole mixture is associated with additional uncertainty. When we compared the IPM risk estimate against the risk estimated using the WMM model in section 3.7.4, we found that the IPM approach underestimated the risk by almost two orders of magnitude. Furthermore, the degree of underestimation was similar when the models were applied to three different situations, smoky coal emissions, coke oven emissions, and MSC versus MSP.

Overall, we estimate that summing the risk attributable to a small number of PAH components in the mixture leads to an uncertainty of up to two orders of magnitude. This relatively low level of uncertainty applies only to our particular study, because we have at least partially validated the IPM model by comparing it to the WMM model. We judge the level of uncertainty in studies by other authors to be higher.

3.9.3 Conclusions

In section 3.9.3, we evaluate the overall uncertainty associated with all the human cancer risk estimates reported in the present document using a non-numerical process. The overall uncertainty takes into account the uncertainty associated with each major step of the dose-response assessment. In general, the overall uncertainty is found to be less than the sum of uncertainty arising from each individual step.

The uncertainty estimates are presented in table 3.9.3.1. The uncertainty is much lower when the WMM model rather than the IPM model is used. The PAH potency estimate for inhalation is more reliable than the potency estimates for other routes of exposure.

Our estimates of uncertainty cannot be compared to the statistical uncertainty reported by a number of authors. Our evaluation process is unique because it places more emphasis on the uncertainty associated with assumptions and judgement made, rather than on the numerical sources of variation, such as the fit of a dose-response function to the data. Under most circumstances, we expect that the uncertainty originating from assumptions and judgement tends to be higher than statistical uncertainty.

Table 3.9.3.1 Estimated level of uncertainty associated with human cancer potency estimates in orders of magnitude.

Extrapolation	Inhalation Primary	Oral Primary	Dermal	Inhalation Secondary	Oral Secondary
WMM to MSP	<<1	1-2	1-2	1	2
B[a]P	<1	2	2	1	2-3
Other PAH	1-2	2-3	2-3	2	3
IPM to MSC	3-4	4	4	4-5	5

3.10 Interpretation of Dose-Response Assessments Based on WMM and IPM

3.10.1 Comparison of WMM and IPM

Either the WMM or the IPM model can be used to estimate the cancer risk in humans from exposure to PAH-containing complex mixtures. It is strongly recommended that both models be used where appropriate, because each model is based on a different set of assumptions and each model estimates the risk of a different segment of the mixture. Section 3.10.2 presents our recommendation for the circumstances under which each risk assessment model can be used and section 3.10.1 presents the rationale for the recommendation.

The IPM model estimates the risk due to only a portion of the mixture. The portion considered consists of the few PAH that human risk has been estimated for. In general, the result represents only a proportion of the potency of the PAH fraction of the whole mixture (see Thorslund and Farrar, 1990 and section 3.7.4) and therefore represents an underestimate of the actual risk due to the PAH in the mixture. On the other hand, the risk estimate covers specific compounds identified and can be applied equally well to mixtures whose PAH composition deviates significantly from the standard reconstituted mixture as described in section 3.4.3.2.2.1 and appendix C. The versatility of the IPM model is the main advantage of the model as compared to the WMM.

The WMM model estimates the risk attributable to the **whole** PAH fraction of a mixture. In the development of the model, it was assumed that the lung carcinogenicity of coke oven emissions was due exclusively to PAH found in the mixture. Although coke oven emissions are rich in PAH, they may also contain other carcinogens. As a result, the risk attributable to PAH only is likely overestimated, but we do not expect the overestimate to be large. For a number of source mixtures, Grimmer and his colleagues (Grimmer *et al.* 1984, 1987, 1987b, 1988; Deutsch-Wenzel *et al.* 1983, 1983b) have shown that the *PAH fraction*, containing PAH with 4 or more rings, accounts for most of the carcinogenic effects for all the mixtures tested. Based on the work of Grimmer and his colleagues as recalculated by Thorslund & Farrar (1990), the overestimation is unlikely to be much larger than 40% of the overall risk of the mixture, and is probably about 10%.

Our analysis shows that the risk estimates derived by using the two models differ by about two orders of magnitude. Much of the difference is likely due to the manner the IPM is applied. The risk of only a small number of PAH present in the mixture is summed up, subsequently, the risk due to the whole PAH fraction is underestimated substantially. On the other hand, the risk obtained with the WMM model is likely greater than the true risk. Therefore, the *true risk attributable to PAH* in a given mixture is expected to be somewhere between the values derived from the WMM and IPM models, and is likely to be much closer to the WMM model.

When the mixture has a profile considerably different from the profile of the standard reconstituted mixture as described in section 3.4.3.2.2.1, the WMM model may not be an acceptable risk assessment model.

3.10.2 When to Use WMM and IPM

We recommend the use of the WMM model as the primary model in most circumstances and simultaneously collecting data to permit the use of the IPM model. The IPM model would be used only when the PAH profile of the mixture deviates unacceptably from that of the MSC. Specifics on determining which of the two risk assessment models to use for a particular mixture are described as follows.

1. Determine the *total risk* for both the MSC and the test mixture. Use only those PAH for which risk values are available and for which the relative contents are available for both the test mixture and the MSC.

2. If the ratio of the "total risks" for the two mixtures is less than 0.1 or greater than 10, use the IPM model, or else use the WMM model.

The principles described can be illustrated using the data in table 3.10.2.1. Table 3.10.2.1 provides an estimate of the potency for MSC and for a hypothetical mixture. The hypothetical mixture has a PAH composition considerably different from that of the MSC, resulting in a different potency

than MSC potency. Since the ratio of the total risk of the MSC to the test mixture is less than 0.1 (actually 0.089), the IPM model is recommended for determining human health risk due to exposure to the hypothetical mixture.

3.10.3 Use of MLE versus UCL

UCL is the upper 95% confidence interval on the initial slope of the dose-response curve. Variation in the initial slope stems strictly from the goodness of fit of a linearized multistage function to the data. Fitting of the data is often a relatively small source of variation compared with other sources. The uncertainty associated with expert judgment often overshadows the uncertainty due to the variation in the data within a data set. Throughout the present report, we have attempted to provide our best estimate in the form of MLEs. UCL slope values and unit risks can be readily derived from MLEs by multiplying the MLE slope by 15.6. This factor represents the ratio of the UCL to the MLE values for the lung cancer risk of coke oven workers as determined by USEPA (1984).

Table 3.10.2.1 Risk associated with MSC and a hypothetical "test mixture".

Compound	Risk (/ng/m^3)	MSC		"Test Mixture"	
		Relative Content	$Risk_{PAH}$ (/ng B[a]P/m^3)	Relative Content	$Risk_{PAH}$ (/ng B[a]P/m^3)
Benzo[a]pyrene	1.5 E-6	1.0	1.5 E-6	1.0	1.5 E-6
Anthanthrene	4.1 E-7	0.31	1.3 E-7	10	4.1 E-6
Anthracene	-	1.6	-	-	-
Benz[a]anthracene	2.1 E-8	1.2	2.5 E-8	1.2	2.5 E-8
Benzo[e]pyrene	0.0	1.1	0.0	0.0	0.0
Benzo[ghi]perylene	1.8 E-8	1.0	1.8 E-8	1.0	1.8 E-8
Benzofluoranthenes	9.4 E-8*	2.5	2.4 E-7	100	9.4 E-6
Chrysene/Triphenylene	3.8 E-8**	2.0	7.6 E-8	2.0	7.6 E-8
Coronene	-	0.34	-	-	-
Dibenz[a,h]anthracene	1.3 E-6	0.28	3.6 E-7	5.0	1.2 E-5
Fluoranthene	-	3.8	-	-	-
Indeno[1,2,3-cd]pyrene	9.9 E-8	0.86	8.5 E-8	0.86	8.5 E-8
Perylene	-	0.45	-	-	-
Phenanthrene	9.5 E-10	4.3	4.1 E-9	4.3	4.1 E-9
Pyrene	0.0	2.8	0.0	0.0	0.0
Acenapthylene	-	0.36	-	-	-
Acenapthene	-	0.71	-	-	-
Fluorene	-	1.6	-	-	-
Total			2.4 E-6		2.7 E-5

* Average of b, j, and k isomers.

** Risk due to chrysene

3.11 References

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APPENDIX A

TUMORIGENICITY OF B[a]P AND 3-MC IN RODENTS

by Pavel Muller

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APPENDIX A TUMORIGENICITY OF B[a]P AND 3-MC IN RODENTS

A.1 Mice

Table A.1.1 Effect of route of administration on the potency of 3-MC in mice

Administration	Dose (mg)	Tumor Site	Incidence	Duration of Treatment	Reference
Drinking w.	abt. 89	forestomach	77.5%	abt. 14 w	Lorenz and Stewart, 1948
Drinking w.	abt. 89	lung	28%	abt. 14 w	Lorenz and Stewart, 1948
p.o.	0.6	lung	63%	single dose, 24 w	Stoner *et al.* 1984
Intratrach. instillation	5	lung squamous carcinoma	15%	10 w	Furst *et al.* 1979
Intratrach. instillation	5	lung adenoma	60%	10 w	Furst *et al.* 1979
Intratrach. instillation	3	lung squamous carcinoma	86%	21-24 w	Nettesheim and Hammons, 1971
Intratrach. instillation	0.1	lung	61%	single, 17 w	Shimkin and Stoner, 1975
Topical application	2.2	skin tumors	100%	20 w	Cavalieri *et al.* 1978

Table A.1.2 Effect of route of administration on the potency of B[a]P in mice

Administration	Dose (mg)	Tumor Site	Incidence	Duration of Treatment	Reference
Diet	20	forestomach	10%	16 w	Neal and Rigdon, 1967
Diet	21-29	forestomach	70%	16-28 w	Neal and Rigdon, 1967
p.o.	4.5	forestomach	60%	6 w	Wattenberg *et al.* 1979
p.o.	0.2	forestomach	45%	single	Pierce, 1961
p.o.	4.5	lung	0%	6 w	Wattenberg *et al.* 1979
p.o.	3	lung	42%	single	Stoner *et al.* 1984
Intratrach. instil.	5	lung adenoma	33%	10 w	Furst *et al.* 1979
Topical	2.5	skin	47%	99 w	Warshawsky and Barkley, 1987
Topical	0.48	skin	57%	48 w	Cavalieri *et al.* 1983
Topical	0.48	skin carc.	43%	48 w	Cavalieri *et al.* 1983

A.2 Rats

Table A.2.1 Effect of route of administration on the potency of 3-MC in rats

Administration	Dose (mg)	Tumor Site	Incidence	Duration of Treatment	Reference
Intubation	960	gastric tumors	0%	80 w	Shay *et al.* 1949
Intubation	960	breast adenoma	60%	80 w	Shay *et al.* 1949
Intubation	270, 670	breast tumors	21, 100%	40 w	Huggins *et al.* 1959
Intubation	2 to 100	breast tumors	10 to 100%	single, 5-19 w	Huggins *et al.* 1961
Intrabronch. pellet	3	lung squam. carcinoma	38	?	Laskin *et al.* 1970
Intralung pellet	0.5	lung squam. carcinoma	44	52 w	Hirano *et al.* 1974
Topical	192	Skin epitheliomas, basal cell carcinomas	100	32 w	Takayma, 1970
Topical	58	Skin basal cell epithelioma	90	26 w	Zackheim, 1964

Table A.2.2 Effect of route of administration on the potency of B[a]P in rats

Administration	Dose (mg)	Tumor Site	Incidence	Duration of Treatment	Reference
p.o.	100	breast	89%	single dose	Huggings and Yang, 1962
Intratrach. instil.	15	Lung squamous carc.	22%	17w	Ishinishi et al., 1979
Intrapulm. implant.	0.1	Lung squamous carc.	31%	22-134w	Wenzel-Hartung et al.,1990
Intrapulm. implant.	0.1	Lung squamous carc.	29%	99-120w	Deutsch-Wenzel et al., 1983

A.3 Hamsters

Table A.3.1 Effect of route of administration on the potency of 3-MC in hamsters

Administration	Dose (mg)	Tumor Site	Incidence	Duration of Treatment	Reference
Intubation	250	forestomach tumors	11.3%	17 w	Homburger *et al.* 1972
Intrabronch. pellet	appx. 0.7	lung squamous carcinoma	61	> 10 w	Hammond *et al.* 1987
Topical	0.5	skin tumors	0	38 w	Shubik *et al.* 1960
Topical	5	skin tumors	0	31 w	Bernfeld and Homburger, 1983

Table A.3.2 Effect of route of administration on the potency of B[a]P in hamsters

Administration	Dose (mg)	Tumor Site	Incidence	Duration of Treatment	Reference
p.o.	20-110	forestomach papill.	7%	4-21 w	Dontenwill and Mohr, 1962
p.o.	130-240	forestomach papill.	46%	26-48 w	Dontenwill and Mohr, 1962
p.o.	20-110	forestomach carcin.	0%	4-21 w	Dontenwill and Mohr, 1962
p.o.	130-240	forestomach carcin.	8%	26-48 w	Dontenwill and Mohr, 1962
p.o.	130-240	skin	0%	26-48 w	Dontenwill and Mohr, 1962
p.o.	130-240	trachea	0%	26-48 w	Dontenwill and Mohr, 1962
Intratrach. instil.	45	lung mostly squamous carc.	100%	15 w	Saffioti et al., 1968
Intratrach. instil.	15	lung squamous carc.	15%	17 w	Yamamoto et al., 1985
Intratrach. instil.	15	lung squamous papil.	31%	17 w	Yamamoto et al., 1985
Intratrach. instil.	4	lung mostly papil.	10%	single dose	Ketkar et al., 1977
Intratrach. instil.	36	lung mostly squamous carc.	66%	36 w	Feron, 1972
Intratrach. instil.	15	lung mostly squamous carc.	30%	30 w	Saffioti et al., 1972
Intratrach. instil.	15	forestomach papil.	7%	30 w	Saffioti et al., 1972
Intratrach. instil.	30	lung mostly squamous carc.	66%	30 w	Saffioti et al., 1972
Intratrach. instil.	30	forestomach papil.	25%	30 w	Saffioti et al., 1972
Intratrach. instil.	60	forestomach papil.	35%	30 w	Saffioti et al., 1972
Intratrach. instil.	60	lung mostly squamous carc.	86%	30 w	Saffioti et al., 1972
Inhalation	127	upper respiratory tract	34%	>= 10 w	Thyssen et al., 1981
Inhalation	127	pharynx	23%	>= 10 w	Thyssen et al., 1981
Inhalation	127	forestomach	4%	>= 10 w	Thyssen et al., 1981
Topical	0.5	skin	0	40 w	Shubik et al.,1960
Topical	5	skin	0	8 w	Bernfeld and Homburger, 1983

A.4 Monkeys

Table A.4.1 Potency of 3-MC in monkeys.

Administration	Dose (g)	Tumor Site	Incidence	Duration of Treatment	Reference
Oral	abt. 1100	any	0%	abt. 5 yrs	Adamson and Sieber, 1983

A.5 Reference

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APPENDIX B

POTENCY OF COMPLEX MIXTURES

by Pavel Muller

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APPENDIX B POTENCY OF COMPLEX MIXTURES

B.1 Potency of Complex Mixtures

Table B.1.1 Potency of complex mixtures.

Type	Mixture	Protocol Summary	Incidence/ng B[a]P	Reference
Coke oven	coke oven - main	initiation	9.4 E-2	Nesnow *et al.* 1982
		complete carc.	6.1 E-4	Nesnow *et al.* 1982
	coke oven - topside	initiation	7.6 E-2	Nesnow *et al.* 1982
Coal-derived	smoky coal particulate - bound	initiation	7.8 E-2	Mumford *et al.* 1990
	smoky coal particulate - bound	complete carc.	2.5 E-4	Mumford *et al.* 1990
	smokeless coal particulate - bound	initiation	2.8 E-2	Mumford *et al.* 1990
	coal tar pitch fumes condensate	initiation	1.1 E-2	Nesnow *et al.* 1982
	coal tar pitch fumes condensate	complete carc.	6.9 E-5	Nesnow *et al.* 1982
	flue gas condensate	complete carc.	6.9 E-4	Grimmer *et al.* 1985
	flue gas condensate	rat intratracheal	1.7 E-2	Grimmer *et al.* 1987
Wood smoke	wood smoke particulate	initiation	5.8 E-2	Mumford *et al.* 1990
	wood smoke particulate	complete carc.	0.0*	Mumford *et al.* 1990
Oil-derived	Nissan diesel	initiation	2.4 E-2	Nesnow *et al.* 1982
	Nissan diesel	complete carc.	0.0*	Nesnow *et al.* 1982
	Oldsmobile diesel	initiation	0.0	Nesnow *et al.* 1982
	Oldsmobile diesel	complete carc.	0.0	Nesnow *et al.* 1982
	Caterpillar diesel	initiation	0.0	Nesnow *et al.* 1982
	Caterpillar diesel	complete carc.	0.0	Nesnow *et al.* 1982
	Mercedes diesel	initiation	B[a]P level NA	Nesnow *et al.* 1982
	VW-Rabbit diesel	initiation	1.1 E-1	Nesnow *et al.* 1982
	reconstituted diesel	rat intratrach.	5.8 E-3	Grimmer, 1987b (see Thorslund draft)

Table B.1.1 Potency of complex mixtures.....continued.

Type	Mixture	Protocol Summary	Incidence/ng B[a]P	Reference
Oil-derived	Mustang exhaust	initiation	5.6 E-2	Nesnow *et al.* 1982
	automobile cond.	complete carc.	3.2 E-4	Grimmer *et al.* 1983; Grimmer, 1985
	automobile cond.	rat intratracheal	1.0 E-2	Grimmer *et al.* 1984
	used oil	complete carc.	1.8 E-4	Grimmer *et al.* 1982
	oil-burning furnace condensate	initiation	B[a]P level NA	Nesnow *et al.* 1982
	reconstituted CSC	rat intratrach.	> 5.0 E-1**	Grimmer, 1988
B[a]P		initiation	4.9 E-3	Nesnow *et al.* 1982
B[a]P		initiation	1.6 E-3	Mumford *et al.* 1990
B[a]P		complete carc.	3.5 E-5	Nesnow *et al.* 1982
B[a]P		complete carc.	1.9 E-4	Mumford et al. 1990
B[a]P		complete carc.	4.1 E-5	Grimmer *et al.* 1982
B[a]P		complete carc.	3.2 E-5	Grimmer *et al.* 1983
B[a]P		complete carc.	8.3 E-5	Grimmer *et al.* 1985
B[a]P		rat intratracheal	2.3 E-4	Grimmer *et al.* 1984
B[a]P		rat intratracheal	2.0 E-4	Grimmer *et al.* 1987
B[a]P		rat intratracheal	2.6 E-4	Grimmer *et al.* 1987b
B[a]P		rat intratracheal	2.6 E-4	Grimmer *et al.* 1988

* Testing was conducted at a dose range probably too low to illicit tumorigenic response. The rationale is presented as follows.

- **Nissan diesel emissions** are very potent in the tumor initiation assay but they were inactive in the complete carcinogenicity assay. We believe that the apparent discrepancy is due to the dose ranges selected in the complete carcinogenesis assay and is therefore a consequence of a flaw in the experimental design.

  In the tumor initiation assay, the tumor incidence for Nissan diesel emissions was about 50% at 2 mg/mouse (Nesnow *et al.* 1982). To achieve about the same level of incidence with coke oven emissions, a dose 20 fold lower was required (0.1 mg/mouse). In the complete carcinogenicity assay, coke oven emissions induced about 30% incidence with 0.5 mg/mouse/week. This is the lowest dose at which the induced tumor rate was clearly distinct from the control rate. Based on the tumor initiation data that Nissan diesel emissions were 20 times less potent than coke oven emissions, one can predict that Nissan diesel emissions would induce 30 % tumor incidence at a dose of 10 mg/mouse/week in the complete carcinogenesis assay. However, similar dose or higher dose levels had not been tested in the study.

- The tumor potency of **wood smoke** is about half the potency of smoky coal or coal tar pitch fumes condensate ("roofing tar") in the tumor initiation assay when potency is expressed in terms of extractable organic content (per mg organics). If the same relative potency can be assumed for the complete carcinogenesis assay, wood smoke would have induced approximately the same level of tumor response as smoky coal and roofing tar at double their doses. In reality, the study had administered wood smoke to the animals at the same dose as smoky coal and at half the dose as roofing tar. It is therefore quite likely that wood smoke would turn out to be an effective tumorigen at about double or quadruple the dose at which it was tested.

** Assuming < 1 ng B[a]P/mg organics (Albert *et al.* 1983)

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APPENDIX C

COMPOSITION OF POLYCYCLIC AROMATIC HYDROCARBONS IN MIXTURES

by Bryan Leece, Devbani Raha, Pavel Muller

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APPENDIX C COMPOSITION OF PAH IN MIXTURES

C.1 Introduction/Objectives

Polycyclic aromatic hydrocarbons (PAH) are released to the environment from a large number of anthropogenic and natural sources. The majority of these sources are combustion processes such as automotive exhaust emissions and forest fires. Non-combustion processes, such as leaching from coal-tar deposits, also release PAH into the environment. Regardless of the source, emissions are complex mixtures of chemicals, consisting of a large number of PAH. Albert *et al.* (1983) suggested that mixtures rather than individual PAH should be the focus of regulation, as exposure to mixture was a better representation of actual exposure. They proposed that the potency of a PAH-containing mixture could be estimated in humans from the known potency of the mixture in an experimental animal species if the potency of another mixture was known in both humans and the same animal species (Albert *et al.* 1983). This approach, known as the relative potency approach, is discussed in detail in the main document. Using this approach, the potency of a mixture can be expressed in terms of the weight of the total extractable organic material (TEO) obtained from a given emission. TEO is defined as the fraction of a sample that can be removed by washing with an organic solvent (usually methylene chloride or cyclohexane). The relative potency approach relies on two important assumptions. 1. The total amount of PAH in the TEO remains constant for a given mixture regardless of the fuel or combustion conditions. 2. The levels of individual PAH relative to a fixed marker (PAH profile) also remain constant. In order to determine the validity of these assumptions upon which the relative potency approach is based, a number of objectives have too be met. These objectives are outlined below.

Objective 1: Determine the differences in total PAH content in different mixtures from the same type of source and among mixtures from different sources.

Objective 2: Determine the effect of changes in operating conditions on the levels of PAH emitted from a source.

Objective 3: Determine the effect of changes in fuel on the levels of PAH emitted from a source.

Objective 4: Determine the similarities or differences in PAH profiles among mixtures from the same type of source and among mixtures from different sources.

Objective 5: Determine the effect of changes in operating conditions on the PAH profiles of mixtures emitted from a single source.

Objective 6: Determine the effect of changes in fuel type on the PAH profiles of mixtures emitted from a single source.

Objective 7: Determine the similarities and/or differences in PAH profiles among mixtures from all sources considered.

Objectives 1 through 3 will be addressed in section C.3.1. Objectives 4 through 7 will be addressed in section C.3.2. The intent of this appendix is not to identify subtle differences in the PAH content or profiles of complex source mixtures, but to determine if the differences which do exist are large enough to affect significantly the risk estimation of mixtures.

C.2 Methods/Data Selection Criteria

Studies which provide analytical data on the levels of individual PAH are available for a number of source mixtures. The mixtures considered have been grouped on the basis of the primary fuel type (coal, wood, petroleum, etc.). Information on the PAH composition of the various mixtures has been obtained from the scientific literature and from government reports. Data were extracted from studies which reported levels of individual PAH in source mixtures with preference given to studies that reported specific levels for B[a]P. Studies which did not report B[a]P levels could not assist in meeting objectives 4 through 7, but could be used, in some cases, to address one or more of the other objectives. Reports that provide information only on the total PAH content or total PAH content plus the levels of one or two PAH were excluded since data of this type are of little use in meeting our objectives.

C.3 Results

C.3.1 Composition of Individual Mixtures

This section determines the differences in PAH emission levels among various source mixtures and, if possible, identifies the effect changes in fuel and operating conditions have on PAH levels (objectives 1 through 3). To address these issues, the levels of individual PAH found in complex source mixtures derived from a number of different types of fuel (coal, wood, petroleum) and a variety of sources were examined. Sections C.3.1.1 through C.3.1.4 describe the fuel types and sources considered while section C.3.1.5 summarizes the composition of PAH for the various sources. Finally, section C.3.1.6 provides a summary of the data and discusses objectives 1 through 3 individually.

C.3.1.1 Coal Derived Source Mixtures

Coal has been and still is used as a source of fuel by a number of industries. It has also been a source of home heating fuel. This section examines the levels of PAH found in the emissions from a number of coal burning sources, including coke ovens, coal-tars (derived from both coking and coal-gasification operations), coal-fired power plants and residential coal-burning stoves. The levels of PAH found in the air at various stages of the steel manufacturing process are also considered. The data are summarized in sections C.3.1.1 through C.3.1.5.

Table C.3.1.1.1.1 Combined gaseous and particulate PAH emissions from coke oven battery tops in spring and fall (Bjøresth *et al.* (1978).

		Spring (μg/m³)			Fall (μg/m³)			
Compound	**# Rings**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Spring/Fall**
Acenaphthylene	3	0	230	-	63	250	130	-
Acenaphthene	3	13	120	50	6.0	24	13	9.2
Fluorene	3	58	150	84	23	140	59	2.5
Fluorene, 9-methyl-,	3	3	81	30	1.7	1200	220	0.37
Fluorene, 2-methyl-,	3	6.6	29	14	2.6	10	5.4	5.4
Fluorene, 1-methyl-,	3	0.7	12	2.0	0.80	4.4	2.1	5.7
Dibenzothiophene	3	16	130	44	5.2	35	15	8.7
Phenanthrene	3	170	960	460	82	490	220	4.4
Anthracene	3	58	330	150	16	160	64	5.2
Carbazole	3	3	23	11	3.1	19	8..8	2.6
Anth/Phen, Methyl	3	39	164	71	10	101	42	3.9
Fluoranthene	4	150	430	282	46	280	140	3.1
Dihydrobenzo[a&b]fluorene	4	20	120	45	9.2	71	33	1.4
Pyrene	4	110	320	210	35	210	110	2.9
Benzo[a]fluorene	4	36	90	56	9.7	71	34	1.6
Benzo[b]fluorene	4	15	61	30	3.1	2.6	13	2.3
Pyrene, 4-methyl-,	4	-	-	-	0	3.0	-	-
Pyrene, 1-methyl-,	4	0	24	10	2.5	17	7	3.4
Benzo[c]phenanthrene	4	6.3	49	19	2.6	13	6.6	2.9
Benz[a]anthracene	4	5.4	156	49	23	110	61	0.80
Chrysene/Triphenylene	4	27	189	76	26	130	68	1.1
Benzo[b,j&k]fluoranthene	5	13	103	40	5.5	104	24	1.7
Benzo[e]pyrene	5	15	73	32	8.0	36	18	1.8
Benzo[a]pyrene	5	24	135	55	14	71	38	1.4
Perylene	5	5.8	19	11	3.3	16	8.2	1.3
Indeno[1,2,3-cd]pyrene	5	9.8	53	25	6.3	75	23	1.1
Benzo[ghi]perylene	6	8.7	23	15	11	45	25	0.60
Anthanthrene	6	6.4	33	14	2.6	62	15	0.93
Dibenzo[a,h]pyrene	6	0	21	11	3.6	14	9.0	1.2
Coronene	7	6.7	24	16	1.0	19	5.3	3.0

C.3.1.1.1 Coke Oven Emissions

Coke is produced by the controlled carbonization of coal. The end use of the coke determines the temperature of the coking operation. The production of coke for the steel industry uses the highest coking temperature (1250°C - 1350°C). Emissions from coke production can arise during the coking operation from a number of places, including coke oven battery top and around the coke oven doors, as well as during the quenching process (WHO, 1985). Data on PAH levels are available for emissions from coke oven battery tops (Bjørseth *et al.* 1978).

Bjørseth *et al.* (1978) reported PAH emissions from coke oven battery tops. The investigators examined the PAH content of both gaseous and particulate emission samples taken during the spring and the fall. The gaseous and particulate emission data have been combined to yield total emissions data for both seasons (see table C.3.1.1.1.1). The mean values in table C.3.1.1.1.1 were derived by summing the mean values for both gaseous and particulate emissions provided by Bjørseth *et al.* (1978). The minimum and maximum values were derived in the same manner. A comparison of the combined data sets for the spring and the fall shows that there is very little difference between the levels in the spring and fall emissions. For the majority of the large molecular weight PAH (≥ four rings), the difference is less than three-fold. For the lower molecular weight PAH (< four rings), the difference in emissions is larger, but is still less than ten-fold (see table C.3.1.1.1.1). Based on the data of Bjørseth *et al.* (1978), the emission levels in the spring are generally higher than those reported in the fall. However, it is not possible to determine if these differences are due to changes in either fuel type or operating conditions. Unfortunately, the summarized data are obtained from a single source. It is unclear whether these results are also representative of emissions from other coke oven sources. Therefore, caution must be exercised in utilizing these data as a model for all coke oven emissions.

C.3.1.1.2 Coal-Tars

Coal tars are produced by the destructive distillation of coal in a process called carbonization or coking. They are generally liquids or semi-solids which contain complex mixtures of PAH, phenols, and heterocyclic compounds containing oxygen, nitrogen and sulphur (WHO, 1985). Greater than 400 individual compounds have been identified in coal tar and some reports suggest that the actual number may reach as high as 10,000 (WHO, 1985). The composition and properties of coal tar depend primarily on the temperature of carbonization. The type of feed stock used plays only a minor role in determining the overall composition of coal tar (WHO, 1985). Due to the dependence of coal tar composition on the temperature of carbonization, coal tars have historically been classified into two types, coal tars derived from low temperature and from high temperature processes (WHO, 1985). Both low temperature (LT) (< 700°C) and high temperature (HT) (> 700°C) coal tars are condensation products, derived from cooling gases

evolved during the carbonization of coal (WHO, 1985). The composition of LT and HT coal tars, and the processes responsible for the generation of each are distinctly different. Therefore, each has to be considered separately.

C.3.1.1.2.1 Low Temperature Coal Tar

Table C.3.1.1.2.1.1 Composition of LTCT from a fixed-bed gasifier.

(all values are rounded to two significant figures)

Compound	mg PAH/g tar	Reference
Naphthalene	6.5	McNeil, 1983
Naphthalene, 1-methyl-,	2.3	McNeil, 1983
Naphthalene, 2-methyl-,	1.9	McNeil, 1983
Acenaphthene	1.9	McNeil, 1983
Fluorene	1.3	McNeil, 1983
Anthracene	0.60	McNeil, 1983
Phenanthrene	16	McNeil, 1983
Carbazole	13	McNeil, 1983
Medium-soft Pitch	260	McNeil, 1983
Fluoranthene	0.18	Griest and Clark, 1982
Pyrene	0.11	Griest and Clark, 1982
Benzo[a]fluorene	0.15	Griest and Clark, 1982
Benzo[b]fluorene	0.10	Griest and Clark, 1982
Pyrene, 3-methyl-,	0.090	Griest and Clark, 1982
Benz[a]anthracene	0.14	Griest and Clark, 1982
Chrysene/Triphenylene	0.090	Griest and Clark, 1982
Benzo[b,j&k]fluoranthene	0.14	Griest and Clark, 1982
Benzo[a]pyrene	0.080	Griest and Clark, 1982
Perylene	0.11	Griest and Clark, 1982

Low temperature coal tars (LTCT) are by-products of coal gasification. They are black viscous liquids which are denser than water. They contain fewer aromatic compounds than HT coal tars. The aromatic compounds found in LTCT are generally alkyl substituted. In addition, LTCT contain higher levels of non-aromatic hydrocarbons and alkali extractable phenolic compounds than HT coal tars (WHO, 1985).

Coal-gasification is the conversion of coal to a gaseous product by the controlled carbonization of coal at low temperatures (< 700°C) in the presence of air, steam and/or carbon dioxide (WHO, 1984). Although coal gasification is no longer prevalent, it was widely used to provide gas for industrial and residential heating and lighting prior to the use of natural gas (WHO, 1984). Fixed-bed, fluidized-bed and entrained-bed gasifiers were the three common processes. Fixed bed gasifiers usually produced greater levels of coal tars than either of the other two processes (WHO, 1984). Temperature, pressure and the level of oxidant all influence the gasification process (WHO, 1984).

McNeil *et al.* (1983) reported the presence of low molecular weight (< four ring) PAH and medium-soft pitch in LTCT (see table C.3.1.1.2.1.1). Medium-soft pitch contains between 40 and 50 percent four to seven ring PAH (WHO, 1984). Griest and Clark (1982) reported the presence of a limited number of higher molecular weight PAH (≥ four rings) in coal tar derived from a fixed-bed coal gasifier (see table C.3.1.1.2.1.1). The data in table C.3.1.1.2.1.1 show that the levels of higher molecular weight PAH are two orders of magnitude lower than those of the lower molecular weight PAH. However, available data are insufficient to determine the effect changes in fuel or operating conditions might have on the composition of LTCT.

C.3.1.1.2.2 High Temperature Coal Tar

High temperature coal tars (HTCT) consist of two main groups, coke oven and continuous vertical retort (CVR) coal tars (WHO, 1985). Production of coke for blast furnaces uses the highest coking temperature (1250°C - 1350°C) while CVR which produces coke for domestic heating and gas production uses a lower temperature (1000°C-1100°C) (WHO, 1985). The difference in operating temperature affects the composition of the respective coal tars. Coke oven tars contain relatively small amounts of aliphatic hydrocarbons compared to CVR tars (WHO, 1985). In addition, the phenolic content of CVR tar is ten fold greater than that found in coke oven tars (WHO, 1985). Both coke oven and CVR tars contain aromatic heterocyclic compounds. In CVR tars, however, the aromatic heterocyclics tend to be alkylated derivatives rather than unsubstituted compounds found in coke oven tars (WHO, 1985).

Table C.3.1.1.2.2.1 PAH composition of various HTCT: Data taken from studies cited in (WHO, 1985). All values are rounded to two significant figures.

	PAH Content (mg PAH/g Tar)						
Compound	**Tar #1[a]**	**Tar #2[b]**	**Tar #3[c]**	**Tar #4[d]**	**Tar #5[e]**	**Tar #6[f]**	**Tar #7[g]**
Acenaphthene	9.6	3.0	11	-	-	6.6	-
Fluorene	8.8	20	7.5	5.1	-	5.0	13
Anthracene	10	18	27	2.6	15	55	12
Phenanthrene	63	57	6	18	50	87	53
Carbazole	13	15	-	8.9	-	-	-
Medium-soft Pitch	600	550	640	440	-	-	-
Pyrene	-	-	-	-	21	99	31
Fluoranthene	-	-	-	-	33	30	40
Benz[a]anthracene	-	-	-	-	6.5	-	8
Chrysene/Triphenylene	-	-	-	-	12	24	11
Perylene	-	-	-	-	2.5	5.5	4.2
Benzo[a]pyrene	-	-	-	-	5.5	4.0	9.7
Benzo[e]pyrene	-	-	-	-	5.0	5.0	6.1
Benzo[a]fluoranthene	-	-	-	-	3.0	-	-
Benzo[b]fluoranthene	-	-	-	-	3.0	-	-
Benzo[j]fluoranthene	-	-	-	-	3.0	-	5.7
Benzo[k]fluoranthene	-	-	-	-	4.0	-	4.9
Dibenz[a,j]anthracene	-	-	-	-	1.0	-	-
Dibenz[a,h]anthracene	-	-	-	-	1.0	-	-
Picene	-	-	-	-	1.5	-	-
Benzo[ghi]perylene	-	-	-	-	5.5	-	7.0
Anthanthrene	-	-	-	-	1.8	-	2.0
Indeno[1,2,3-cd]pyrene	-	-	-	-	5.0	-	8.0
Coronene	-	-	-	-	-	-	2.0
Methyl Phen/Anth	-	-	-	-	-	-	7.5
Benzo[a&b]fluorene	-	-	-	-	-	-	7.3
Benzo[c]phenanthrene	-	-	-	-	-	-	1.7

Table C.3.1.1.2.2.1 PAH composition of various HTCT: Data taken from studies cited in (WHO, 1985).....continued. All values are rounded to two significant figures.

Compound	PAH Content (mg PAH/g Tar)						
	Tar #1[a]	Tar #2[b]	Tar #3[c]	Tar #4[d]	Tar #5[e]	Tar #6[f]	Tar #7[g]
Benzo[ghi]fluoranthene	-	-	-	-	-	-	1.5
Cyclopenta[cd]pyrene	-	-	-	-	-	-	0.63
Benz[a]aceanthrylene	-	-	-	-	-	-	7.9
Pentaphene	-	-	-	-	-	-	0.4
Benzo[b]chrysene	-	-	-	-	-	-	0.6
Reference	1	1	1	1	2	3	4

a) Coal tar from U.K. Coking
b) Coal Tar from German Coking
c) Coal tar from U.S. Coking
d) CVR tar
e) HTCT type unspecified
f) Coal Tar from coking Location not stated
g) National Bureau of Standards Medium Crude Coke Oven Tar

1) McNeil, 1983
2) Kleffner *et al.* 1981
3) Novotny *et al.* 1981
4) Nishioka *et al.* 1986

The PAH composition of various HTCT is presented in table C.3.1.1.2.2.1. For four of the seven tars listed, only the levels of the lower molecular weight PAH are reported. The remaining PAH are identified as *Medium Soft Pitch*. Table C.3.1.1.2.2.1 reports the levels of both the lower and higher molecular weight PAH for the other three HTCT. The data show moderate variation in the levels of anthracene and phenanthrene. The highest levels are about 21 fold and 15 fold higher than the lowest levels reported for anthracene and phenanthrene, respectively. Differences between the highest and lowest levels are all less than five-fold for other PAH. Pyrene is the only higher molecular weight PAH (≥ four rings) whose highest level is more than three-fold (approximately five fold) greater than the lowest level reported. The fact that the coal-tar samples listed in table C.3.1.1.2.2.2 come from a number of coking operations and that the apparent difference in PAH levels is small suggests that fuel-type and operating conditions may not have a significant effect on the levels of PAH found in HTCT. This feature appears to be particularly true for the larger molecular weight PAH.

C.3.1.1.2.3 Comparison of Low and High Temperature Coal tars

A comparison of the PAH content of both LTCT and HTCT shows that the levels of all PAH are higher in the HTCT than in the LTCT. The differences are greatest for the four to seven ring PAH whose levels in HTCT are at least an order of magnitude higher than those found in LTCT (see tables C.3.1.1.2.1.1 and C.3.1.1.2.2.1). The significance of these differences will be discussed in detail in section 3.1.5.

C.3.1.1.3 Iron and Steel Foundries

Polycyclic aromatic hydrocarbons can be released during the manufacture of iron and steel. The production of coke is a major source but other stages in the process may also serve as sources of PAH emissions, such as casting as well as pyrolysis of carbon containing loading materials in the molding sand (Knecht *et al.* 1986). Knecht *et al.* (1986) examined the PAH content of dust and air samples taken in the casting area of an iron foundry. The study reported the levels of 20 PAH in both gaseous and particulate phases of air samples taken near where castings were shaken from the molds. The total PAH levels (particulate and gaseous phases) are shown in table C.3.1.1.3.1. A comparison of the total gaseous and particulate PAH levels released during casting versus PAH levels from the battery top of a coking operation (see section C.3.1.1.1) shows that the emission levels from coke ovens are approximately two orders of magnitude greater than those released during the casting operations. However, the data are insufficient to evaluate the effect of differences in moulding sand and casting operations on PAH emission levels.

C.3.1.1.4 Coal-Fired Power Plants

The burning of coal in power plants does not usually achieve complete combustion of coal to carbon dioxide and water (Warman, 1985). This incomplete combustion can be a source of PAH emissions. Emissions from coal-fired power plants are dependent upon a number of factors, including the size of the plant, the loading factor, the type of coal, the type of burner and the flue-gas cleaning equipment (Warman, 1985). Both the loading factor and the type of burner are thought to be the most crucial factors (Warman, 1985). For example, it has been suggested that stoker fired installations produce higher weight per volume emissions than facilities which use front or tangentially fired systems (Warman, 1985). However, the available data are insufficient to determine the effects that each of the factors listed above has on PAH emissions.

Table C.3.1.1.3.1 Total PAH levels in air samples from casting operations (Knecht *et al.* 1986).

(all values are rounded to two significant figures)

Compound	Total PAH ($\mu g/m^3$)
Phenanthrene	4.5
Anthracene	2.3
Fluoranthene	1.6
Pyrene	1.7
Benzo[a]fluorene	0.48
Benzo[b]fluorene	0.41
Benzo[b]naphtho[1,2-d]thiophene	0.10
Benzo[ghi]fluoranthene	0.15
Benz[a]anthracene	0.67
Chrysene/Triphenylene	0.82
Benzo[b,j&k]fluoranthene	0.87
Benzo[e]pyrene	0.48
Benzo[a]pyrene	0.47
Perylene	0.21
Dibenz[a,h]anthracene	0.24
Indeno[1,2,3-cd]pyrene	0.81
Dibenzo[a,h]pyrene	0.20
Benzo[ghi]perylene	0.72
Anthanthrene	0.64
Coronene	0.21

Large power plants (> 500 MW) are generally fueled with pulverized coal using front, tangential or opposed firing systems (Warman, 1985). On the other hand, smaller plants (< 500 MW) more often use non-pulverized coal in conjunction with surface fed, stoker or spreader-stoker firing systems (Warman, 1985). Other common methods of coal burning include moving grate and cyclone furnaces, and fluidized bed combustors. There are three large coal-fired power plants currently in operation in the province of Ontario, Nanticoke (4000 MW), Lakeview (2400 MW) and Lambton (2000 MW). All the three plants use pulverized coal with either front or tangential firing. Flue-gas emissions from coal-burning power plants using pulverized coal, moving grate or fixed grate or fluidized bed firing in Scandinavia and the United States are summarized in tables C.3.1.1.4.1 and C.3.1.1.4.2.

Table C.3.1.1.4.1 summarizes the emission data from the Scandinavian power plants. Warman (1985) indicated that the data for "*Plant H*" were collected during a period when emissions were uncontrolled. Therefore, the plant "H" data have not been considered further. The remaining data show variation in PAH emission levels among plants that use the same firing systems and among plants that use different firing systems. Warman (1985) suggested that differences in sampling methodologies might be, in part, responsible for these observed differences. The *Plant A & B* data were collected using one method while *Plants C - I* data were collected using a different methodology. A third sampling technique was used for *Plant J*. Among the PAH emission data collected using a single sampling method, (*C* through *I*) the data from the fixed grate plant (*Plant G*) appear to be the highest. PAH emissions from the fluidized bed plant (*Plant I*) are lower than those from *Plant G*, but higher than those from either the pulverized coal (*Plant C*) or the moving grate plants (*Plants D - F*) (see table C.3.1.1.4.1). The level of emissions from the pulverized coal and moving-grate plants do not appear to be different. However, all PAH are reported at or below the minimum detection limit (MDL) for the two plants. Therefore, it is not possible to properly identify real similarities/differences between the two systems. *Plant A* PAH emissions (collected with a different sampling technique from that used for *Plant C*) are not much different from emissions from *Plant B*. The differences in PAH levels between *Plants A & B* and *Plant C* likely result from a difference in the sampling method (Warman, 1985).

The data in table C.3.1.1.4.1 indicate that differences in operating conditions (*Plants C - I*) can have an effect on the levels of PAH emitted. A lack of pollution control results in the highest PAH levels (*Plant H*). With pollution control in place, the highest levels are observed at the fixed-grate plants, while the lowest PAH levels are seen at both the pulverized coal and moving-grate plants. Within the same plant type, differences in PAH emissions among different plants appear to be small.

Table C.3.1.1.4.1 PAH in flue-gas from Scandinavian coal-fired power plants (data from Warman (1985)).

(all values are rounded to two significant figures)

	PAH Levels in Flue-Gas Emissions (µg PAH/m³ Air Sampled)									
	Pulverized Coal			Moving-Grate			Fixed Grate	Fluidized-Bed		
Compound	Plant A	Plant B	Plant C	Plant D	Plant E	Plant F	Plant G	Plant H	Plant I	Plant J
Phenanthrene	0.37	0.85	<0.10	<0.10	<0.10	0.29	15	660	17	<1.0
Anthracene	0.040	0.050	<0.10	<0.10		<0.10	<0.10	110	0.90	-
Methyl Phen/Anth	0.25	0.28	-	<0.10		-	<0.10	16	0.71	-
Fluoranthene	0.80	0.38	<0.10	<0.10	<0.010	<0.10	22.1	230	5.7	<1.0
Pyrene	0.31	0.18	<0.10	<0.10	<0.070	<0.10	0.91	210	2.9	<1.0
Pyrene, 4-methyl-,	<0.020	<0.020	-	-	-	-	-	-	-	-
Pyrene, 1-methyl-,	<0.020	<0.020	-	-	-	-	<0.10	-	-	-
Benz[a]anthracene	0.080	0.040	<0.10	<0.10	-	<0.10	1.4	32	<0.10	<0.6
Chrysene/Triphenylene	0.37	0.19	<0.10	<0.10	<0.010	<0.10	<0.10	18	<0.10	<0.6
Benzo[b]fluoranthene	0.15	0.030	-	-	-	-	0.88	26	-	-
Benzo[k]fluoranthene	0.080	0.020	-	-	-	-	1.4	-	-	-
Benzo[ghi]fluoranthene	-	-	<0.10	<0.10	<0.010	<0.10	0.81	23	<0.10	-
Benzo[e]pyrene	0.080	0.010	<0.10	<0.10	<0.010	<0.10	0.79	13	<0.10	-
Benzo[a]pyrene	0.070	0.020	<0.10	<0.10	<0.010	<0.10	<0.10	13	<0.10	<0.2
Indeno[1,2,3-cd]pyrene	-	<0.010	<0.10	<0.10	<0.010	<0.10	<0.10	5.0	<0.10	-
Perylene	-	<0.010	<0.10	<0.10	<0.010	<0.10	<0.10	2.1	<0.10	-
Benzo[ghi]perylene	-	<0.010	<0.10	<0.10	<0.010	<0.10	<0.10	3.9	<0.10	-
Benz[c]acenaphthylene	-	-	-	-	-	-	<0.10	-	-	-
Benz[e]acenaphthylene	-	-	-	-	-	-	-	22	-	-
Benzofluorenes	-	-	-	-	-	-	<0.10	-	-	-
Dibenz[a,h]anthracene	-	-	-	-	-	-	<0.10	1.0	-	-
Coronene	-	-	-	-	-	-	<0.10	-	-	-
Dimethyl Phen/Anth	-	-	-	-	-	-	-	8.9	-	-
Trimethyl Phen/Anth	-	-	-	-	-	-	-	1.9	-	-
Benzo[c]phenanthrene	-	-	-	-	-	-	-	14	-	-
Dibenzo[a,c]fluorene	-	-	-	-	-	-	-	20	-	-
Dihydrobenz[a]anthracene	-	-	-	-	-	-	-	4.1	-	-
Benzo[a]fluoranthene	-	-	-	-	-	-	-	7.5	-	-
References	1	1, 2, 3	4	5, 6	7, 8	9	10	7, 11	12	?

1) Eriksson, 1980
2) Eklund & Strömberg, 1981a
3) Kveseth, 1981a
4) Nilsson, 1982
5) Eklund & Strömberg, 1981b
6) Kveseth, 1981b
7) Eklund, 1981
8) Kveseth, 1981c
9) Nyström, 1982
10) Eklund & Strömberg, 1982
11) Trzcinski, 1981
12) Andersson, 1982

Table C.3.1.1.4.2 PAH in flue-gas from U.S. coal-fired power plants (data from Bennet *et al* (1979)).

(all values are rounded to two significant figures)

Compound	Emissions (μg PAH/m³ Air)		
	Plant 1	Plant 2	Plant 3
Phen/Anth	0.018	0.24	0.22
Anthracene, methyl-,	0.0022	0.014	0.046
Fluoranthene	0.0019	0.015	0.060
Pyrene	0.00040	0.011	0.032
Methyl Pyr/Fluoran	<0.00010	0.0070	0.013
Benzo[c]phenanthrene	<0.00010	<0.00020	<0.00020
Chrysene/Benz[a]anthracene	<0.00010	<0.00020	<0.0064
Chrysene, methyl-,	<0.00010	<0.00020	<0.00020
Benz[a]anthracene, 7,12-dimethyl-,	<0.00010	<0.00020	<0.00020
Benzofluoranthenes	<0.00010	<0.00020	0.00030
Benzo[a]pyrene	<0.00010	<0.00020	0.00030
Benzo[e]pyrene	<0.00010	-	-
Perylene	<0.00010	0.00080	0.0022
Benzopyrenes, methyl-,	<0.00010	<0.00020	<0.00020
Cholanthrene, 3-methyl-,	<0.00010	<0.00020	<0.00020
Indeno[1,2,3-cd]pyrene	<0.00010	<0.00020	<0.00020
Benzo[ghi]perylene	<0.00010	<0.00020	<0.00020
Dibenz[a,h]anthracene	<0.00010	<0.00020	<0.00020
Dibenzo[c,g]carbazole	<0.00010	<0.00020	<0.00020
Dibenzo[a,i & a,h]pyrenes	<0.00010	<0.00020	<0.00020
Coronene	<0.00010	<0.00020	<0.00020

Table C.3.1.1.4.2 summarizes the emissions from coal-fired power plants in the United States. All three plants were equipped with electrostatic precipitators and the same sampling and analysis techniques were used at each site (Bennett *et al.* 1979). However, the authors had not reported the types of firing systems used in each plant (Bennet *et al.* 1979). The PAH levels are

at or below the MDL at all three plants and thus, comparison among the three plants is difficult. For the few PAH for which levels have been reported, the emissions from *Plant 1* appear to be lower than those from *Plants 2 & 3*. Differences in PAH emissions between *Plants 2* and *3* also appear to be limited. However, as the types of firing systems and coal used have not been identified, it is not possible to determine what effects these factors have on PAH emission from these plants.

In general, coal-burning power plants, operating under normal conditions, produce very low levels of PAH. It is only when the plant operations are faulty or uncontrolled do PAH emission levels appear to rise (Warman, 1985). The data suggest that operating conditions (type of firing system used) can have an effect on the levels of PAH emissions and that the type of firing system must be considered for risk estimation. However, as the types of coal used were not specified by the authors, it is not possible to evaluate what effect changes in fuel type would have on PAH emissions.

C.3.1.1.5 Residential Coal Stoves

Brockhaus and Tomingas (1976) examined the effect of fuel type on the PAH emissions from a "*Universal Slow Combustion*" stove. The results are summarized in table C.3.1.1.5.1. A number of different fuel types were used including anthracite (Ant), anthracite briquettes (A.B.), lignite briquettes (L.B.), Broken coke (B.C.) and two special fuels "*Anzit*" and "*Extrazit*". There is a marked difference in the emission levels among the various fuel types. Lignite and anthracite briquettes produce PAH at levels two to four orders of magnitude greater than other fuels (see table C.3.1.1.5.1). Lignite, or brown coal, is a soft coal which has higher moisture and lower fixed carbon content than anthracite coal (Brockhaus and Tomingas, 1976). It burns at a lower temperature than anthracite, resulting in less complete combustion of the organic matter and produces a smoky flame (Brockhaus and Tomingas, 1986). Consequently, lignite is expected to produce higher levels of emissions than other coal sources. Anthracite briquettes produced the highest levels of emissions of all the fuels tested. Emissions from anthracite briquettes were three to four orders of magnitude higher than those of anthracite. The authors indicated that anthracite briquettes were formed when coal-tar was used as a binding material (Brockhaus and Tomingas, 1976). This characteristic could account for the very high emission levels from burning anthracite briquettes as compared with burning anthracite. Anthracite, broken coke, *Anzit* and *Extrazit* all produced relatively low levels of PAH. Anthracite and broken coke are relatively clean fuels that burn with a smokeless flame. Because they burn at higher temperatures than lignite, the combustion of the organic material is more complete. As a result, they can be expected to produce fewer emissions than lignite. The authors identified *Anzit* and *Extrazit* as

special coals, but provided no further description of their nature. Based on the data in table C.3.1.1.5.1, these fuels seem to resemble anthracite and broken coke. All in all, the data show that fuel type can have a marked effect on the emission of PAH for a given type of coal stove.

Table C.3.1.1.5.1 PAH emissions from different fuels in a coal-burning stove (Brockhaus and Tomingas, 1976).

(all values are rounded to two significant figures)

	Fuel Type (PAH Emissions µg PAH/m^3 Air Sampled)					
Compound	**A.B.**	**L.B.**	**Ant**	**B.C.**	***Anzit***	***Extrazit***
Fluoranthene	380	70	0.087	-	3.9	0.028
Pyrene	87	15	0.025	0.0017	0.13	0.0044
Benz[a]anthracene	160	21	0.069	0.0087	1.8	0.0045
Chrysene	160	16	0.11	0.019	3.5	0.018
Benzo[b]fluoranthene	210	15	0.16	0.071	1.9	0.016
Benzo[k]fluoranthene	67	3.2	0.027	0.0067	0.026	0.00060
Benzo[e]pyrene	200	34	0.19	1.2	0.68	0.032
Benzo[a]pyrene	65	5.0	0.012	0.010	0.0022	0.00050
Perylene	140	13	0.023	0.017	0.14	-
Dibenz[a,h]anthracene	23	10	0.013	-	0.054	-
Benzo[ghi]perylene	96	20	0.027	0.12	0.073	0.0051
Coronene	41	6.3	0.0076	0.0058	0.011	0.0016

Brockhaus and Tomingas (1976) had monitored the effects of fuel type on PAH emissions using a single type of stove. Grimmer *et al.* (1983), on the other hand, monitored PAH emissions from brown coal burned in two different types of stoves, a "*Slow Combustion Stove (SCS)*", and a "*Permanent Combustions Stove (PCS)*". Their objective was to examine the effects of stove type on PAH emissions (Grimmer *et al.* 1983)). The data (see table C.3.1.1.5.2) show that the PAH emissions from PCS are higher than those from the SCS. However, the differences are all less that five-fold, suggesting that stove type has only a small effect on PAH emissions.

Table C.3.1.1.5.2 Effect of stove type on PAH emissions from coal stoves (Grimmer *et al.* 1983).

(all values are rounded to two significant figures)

	PAH Emissions (mg PAH/kg Fuel Burned)						
	SCS (A)			PCS (B)			
Compound	S1	S2	Mean	S1	S2	Mean	B/A
Fluoranthene	3.3	3.4	3.3	17	14	15	4.5
Pyrene	3.0	3.0	3.0	12	11	12	3.8
Benz[a]anthracene	1.0	1.2	1.1	3.7	3.3	3.5	3.2
Chrysene/Triphenylene	1.4	1.8	1.6	5.6	5.2	5.4	3.4
Benzofluoranthenes	1.0	0.90	0.95	3.2	2.9	3.0	3.2
Benzo[a]pyrene	0.43	0.50	0.46	1.3	1.2	1.2	2.6
Benzo[e]pyrene	0.58	0.40	0.48	1.7	1.5	1.6	3.3
Perylene	0.21	0.20	0.20	0.50	0.40	0.44	2.2
Indeno[1,2,3-cd]pyrene	0.23	0.20	0.21	0.50	0.60	0.55	2.6
Benzo[ghi]perylene	0.41	0.30	0.35	0.50	0.50	0.50	1.4
Anthanthrene	0.050	0.030	0.040	0.080	0.080	0.080	2.0

The data presented in tables C.3.1.1.5.1 and C.3.1.1.5.2 indicate that both the type of fuel and the type of stove can have an effect on the levels of PAH emitted from residential coal-burning stoves. Of the two factors, fuel type has a much greater (2 to 4 orders of magnitude) effect than stove type (less than five-fold). The magnitude of the effect implicates that differences in PAH emissions arising from changes in the type of stove used are unlikely to have an impact on the assessment of risk. On the other hand, changes in the type of fuel may have a significant effect on risk assessment. Therefore, it may be possible to treat emissions from various stove burning the same fuel as equivalent without seriously affecting risk estimation. The same cannot be assumed for situations where different fuels are used. It should be noted that the data sets upon which these conclusions are based are small. Therefore, one can place only limited confidence on these conclusions.

C.3.1.1.6 Coal Derived Source Mixtures: Summary

Sections C.3.1.1.1 through C.3.1.1.5 have reviewed PAH emission levels from a number of sources which use coal as the fuel. Direct comparison of the levels emitted from various sources is complicated by differences in sampling techniques and reporting formats. For example, the location from which a sample is taken affects the measurement. Samplers placed directly at the source could be expected to register higher PAH levels on a $\mu g/g$ particulate or $\mu g/m^3$ basis than a sampler removed from the immediate source. While reliable quantitative comparisons are not really possible, some semi-quantitative estimations can be made and are summarized below.

- Among the various sources (coke ovens, iron and steel foundries, oil-fired power plants and residential coal-burning stoves) considered, PAH emission levels from coke ovens appear to be the highest on a $\mu g/m^3$ basis. With the exception of anthracite briquette emissions from coal stoves, PAH emissions from the other three sources are generally lower than those from coke ovens.
- Operating conditions have an effect on the levels of PAH detected. The levels of PAH detected in coal-tars seem to be dependent on the process (LT or HT) by which the tars are generated (see section C.3.1.1.2). While differences in the firing systems used at coal-fired power plants alter the levels of PAH emitted (see section C.3.1.1.4), the type of stove has only a small effect on PAH emissions (see section C.3.1.1.5).
- Information on the effect of fuel type on the emissions of PAH from coal burning sources is limited to coal stoves. In general, fuel type can have a marked effect on the levels of PAH emitted from a single type of coal burning stove (see section C.3.1.1.5.

In summary, the available data indicate that both the operating conditions and the type of fuel used can alter the levels of PAH emitted from a source. The available data are insufficient to fully evaluate the effects that each of these parameters has on PAH emissions.

C.3.1.2 Wood Derived Source Mixtures

Section C.3.1.2 examines emissions from sources that use wood as a fuel. For the purpose of appendix C, these sources are grouped into two classes, open burning sources and wood-burning stoves. Open burning sources include forest fire, residential open fireplaces and open burning of wood waste materials.

C.3.1.2.1 Forest and Open Fires

Freemann and Cattell (1990) have monitored PAH emissions from a simulated residential fireplace, an open backyard fire and a bush fire. The results are shown in table C.3.1.2.1.1. The simulated residential fireplaces burned only wood, while the open fire burned wood and leaves. Emission data for the bush fire were obtained from a large uncontrolled burn of native vegetation near Sydney, Australia. The data in table 3.1.2.1-1 show that there are slight differences in PAH emissions among the three types of open wood burning fires.

Emissions from residential fireplaces, bush and open fires do not appear to differ significantly from each other. The lowest and the highest reported levels for a majority of the PAH differ by less than five-fold (see table C.3.1.2.1.1). The largest variations are observed for coronene, dibenz[a,h]anthracene and B[a]P, where the differences between the highest and lowest reported values are 37, 10 and 7, respectively. It is likely that differences in the location of the samplers for each of these sources may account for some of the observed differences. Without further information, it is difficult to fully evaluate the differences that fuel type and burn conditions are likely to have on PAH emissions. However, the little difference in the reported levels suggests that differences in the fuel type and burn conditions are likely to have only small effects on the levels of PAH emitted for these types of sources.

McMahon and Tsoukalas (1978) monitored PAH emissions from two types of simulated forest fires, heading and backing fires. Heading fires are fires which spread with the wind. They are characterized by a fast rate of spread, a wide burn area and a long flame. Backing fires are fires which burn against the wind, characterized by a slow spread, a narrow burn area and a small flame (McMahon and Tsoukalas, 1978). The study simulated the natural arrangement of fuel on the forest floor by using slash pine needles as the test fuel. Burns were conducted on a four foot by three foot inclined burn grate. Heading fires were simulated by allowing the fire to progress up slope. Samples were taken during the smoldering and flaming phases of the burn. The study simulated backing fires by starting the burn at the top of the incline and allowing the fire to progress down slope. Samples were taken over the entire burn period. Three different fuel loadings were tested in the study. The results are summarized in table C.3.1.2.1.2.

The data show that the PAH emissions from backing fires are greater than those from heading fires. For both types of fires, increasing the fuel loading decreases the levels of PAH emissions. Emission levels vary by as much as two orders of magnitude between heading and backing fires of the same fuel loading. When all three fuel loadings are compared, PAH emissions vary by as much as three orders of magnitude. McMahon and Tsoukalas (1978) noted that the slash pine needle system was an incomplete model of the forest floor as the fuel used in the tests had a uniform moisture content. In reality, the fuel on the forest floor is expected to have varying moisture content and fuel loadings. These factors may have an effect on PAH emissions.

Table C.3.1.2.1.1 PAH emissions from open wood-burning sources.

(all values are rounded to two significant figures)

Compound	# of Rings	Emissions (μg PAH/g Particulate) Fireplace (A)	Bush Fire (B)	Open Fire (C)	Ratio A:B:C
Fluoranthene	4	88	70	200	1 : 0.80: 2.3
Pyrene	4	100	68	130	1 : 0.67: 1.3
Benz[a]anthracene	4	39	60	38	1 : 1.5: 0.98
Chrysene	4	63	64	130	1 : 1.0: 2.1
Benzo[b]fluoranthene	5	30	6.2	6.0	1 : 0.21 : 0.20
Benzo[k]fluoranthene	5	21	10	21	1 : 0.48 : 1
Benzo[a]pyrene	5	27	71	190	1 : 2.6: 7
Dibenz[a,h]anthracene	5	1.2	4.3	12	1 : 3.6: 10
Indeno[1,2,3-cd]pyrene	5	10	20	20	1 : 2 : 2
Benzo[ghi]perylene	6	5.8	10	8.2	1 : 1.7 : 1.4
Coronene	7	0.70	1.0	26	1 : 1.4 : 37

A comparison of PAH emission levels for all fires shows that emissions from backing fires are substantially higher than those from other types of fires (see table C.3.1.2.1.1 and C.3.1.2.1.2). Based on the data from heading and backing fires, one can conclude that fuel-loading and combustion conditions can have an effect on the levels of PAH emitted. Changes in the fuel type and combustion conditions can also affect the levels of PAH emitted for residential fire-places, bush and open fires. However, the available data are insufficient to determine the effect of each individual parameter.

Table C.3.1.2.1.2 PAH emissions from simulated forest fires.

(all values are rounded to two significant figures)

	PAH Emissions (µg PAH/g Particulate Matter)					
	Simulated Backing Fires			Simulated Heading Fires		
Compound	0.1 lb/ft²	0.3 lb/ft²	0.5 lb/ft²	0.1 lb/ft²	0.3 lb/ft²	0.5 lb/ft²
Phenanthrene/Anthracene[1]	1100	490	230	250	150	110
Anthracene, methyl-,	850	250	180	110	130	130
Fluoranthene	1300	480	270	73	26	18
Pyrene	1900	690	430	110	26	19
Pyr/Fluoran, methyl-,[2]	1700	550	490	73	45	42
Benzo[c]phenanthrene	800	400	190	24	3.8	3.0
Chrysene/Benz[a]anthracene	2600	1200	810	58	15	14
Chrysene, methyl-,	1600	420	350	58	35	26
Benzofluoranthenes	1200	270	330	16	3.5	4.1
Benzo[a]pyrene	310	120	95	38	1.1	1.6
Benzo[e]pyrene	530	260	270	61	2.1	2.6
Perylene	190	44	54	33	6.5	0.78
Benzopyrenes, methyl-,	600	210	150	65	5.4	11
Indeno[1,2,3-cd]pyrene	390	150	68	-	-	-
Benzo[ghi]perylene	560	220	170	-	-	-

1) Mixture of phenanthrene and anthracene (ratio not stated).

2) Methylated pyrenes and fluoranthenes. Sites of methylation not stated.

C.3.1.2.2 Residential Wood Burning Stoves

Ramdahl *et al.* (1982) monitored PAH emissions from an air-tight stove burning either hardwood (birch) or softwood (spruce) under "*normal*" and "*air-starved*" conditions. *Normal* operations were defined as conditions in which enough air was provided so that the wood burned with a flame. *Air-starved* conditions limited the air supply so that the wood burned without a flame. The results are summarized in table C.3.1.2.21. The data show that birch, burned under *normal* conditions, appears to produce the highest levels of emissions. However, the authors noted that the *normal* birch samples were taken following an air-starved test and that the stove stack had not been cleaned prior to monitoring. The authors suggested that these high readings were due, in part, to the volatilization of creosote which had condensed on the stack wall from a previous test. Considering this shortcoming, one should view the PAH emission data from the birch *normal* burn with caution. Comparing PAH levels emitted from burning birch and burning spruce under *air-starved* conditions shows that the emissions from spruce are higher than those from birch, ranging between 1.8 and 10-fold (see table C.3.1.2.2.1). Thus, the type of wood burned would seem to have an effect on the levels of PAH emitted. Burning of spruce under *normal* conditions produced fewer PAH than *air-starved* burning. This characteristic suggests that operating conditions can alter the amount of PAH released from wood-burning stoves. The data presented show that the levels of PAH emitted from wood-burning stoves can be affected by both the fuel and the operating conditions.

The effect of stove and fuel type on PAH emissions from wood-burning stoves was studied by Peters *et al.* (1981) who monitored PAH emissions from baffled and non-baffled stoves burning oak and pine. The data, provided as g PAH per kg of fuel burned, are summarized in table C.3.1.2.2.2. Both baffled and non-baffled stoves were operated under "normal" conditions. Seasoned or dry pine was burned in the baffled stove and green pine was burned in the non-baffled stove. The data show that emissions from the two types of stoves are very similar for a given type of wood. In addition, PAH emissions from burning the pine fuel are greater than those from burning oak in both types of stoves. Although the emissions of individual PAH are higher for pine fuel than for oak, the differences are less than three fold for all PAH with the exception of dibenzphenanthrenes and dibenzanthracenes when both were burned in the non-baffled stove. These data suggest that, provided the stoves are operated under the same conditions, the type of stove used has only a marginal effect on PAH levels.

Table C.3.1.2.2.1 PAH emissions from air-tight wood burning stoves.

(all values are rounded to two significant figures)

	PAH Emissions (μg PAH/m³ Air)			
	Birch		Spruce	
Compound	Normal	Starved	Normal	Starved
Phenanthrene	620	290	96	780
Anthracene	87	95	14	170
Methyl Phen/Anth	190	290	52	590
Fluoranthene	300	76	34	300
Benz[e]acenaphthylene	73	24	8.5	140
Pyrene	290	85	27	350
Benzo[a]fluorene	16	30	2.1	78
Benzo[b]fluorene	18	22	1.3	-
Pyrene, 4-methyl-,	10	23	-	-
Pyrene, 2-methyl-,	11	48	-	88
Pyrene, 1-methyl-,	-	34	-	76
Cyclopenta[cd]pyrene	41	9.7	4.6	67
Benz[a]anthracene	51	19	6.9	72
Chrysene/Triphenylene	65	27	-	77
Benzo[b]fluoranthene	110	10	12	94
Benzo[e]pyrene	37	9.5	4.0	32
Benzo[a]pyrene	60	14	5.3	57
Perylene	29	1.4	2.6	3.5
Indeno[1,2,3-cd]pyrene	44	9.7	3.8	27
Benzo[ghi]perylene	58	11	3.9	26

Quantitative comparison between the work of Ramdahl *et al.* (1982) and Peters *et al.* (1981) is not possible because their PAH emission data were not provided in comparable formats. However, on a qualitative basis, the pattern of emissions would seem to be the same. Softwood (spruce and pine) tends to produce greater levels of PAH emissions than hardwood (birch and oak) when burned under the same conditions. The differences are usually less than ten-fold. It

is not possible to compare the emissions from air-tight stove used by Ramdahl *et al.* (1982) with those from baffled and non-baffled stoves used by Peters *et al.* (1981) because of different data reporting formats. However, the work of Peters *et al.* (1981) suggests that stove type has little effect on the levels of PAH released provided that the stoves are operated under similar conditions. In addition, the work of Ramdahl *et al.* (29) shows that operating conditions can affect the levels of PAH emitted. Therefore, although the effect of fuel and operating conditions, each on its own, appears to be usually less than ten-fold, both should be considered when examining PAH emissions from residential wood-burning stoves.

Table C.3.1.2.2.2 PAH emissions from baffled and non-baffled wood burning stoves.

(all values are rounded to two significant figures)

	PAH Emissions (g PAH/kg Fuel)			
	Baffled Stove		Non-Baffled Stove	
Compound	**Oak**	**Dry Pine**	**Oak**	**Green Pine**
Phenanthrene/Anthracene	0.075	0.15	0.062	0.10
Methyl Phen/Anth	0.021	0.051	0.017	0.051
Fluoranthene	0.018	0.032	0.021	0.019
Pyrene	0.016	0.024	0.017	0.019
Methyl Fluoran/Pyr[1]	0.013	0.017	0.010	0.014
Benzo[ghi]fluoranthene	0.0048	0.0067	0.0047	0.0047
Cyclopenta[cd]pyrene	0.0048	0.0089	0.0051	0.014
Benzo[c]phenanthrene	0.0016	0.0023	0.0016	0.0046
Benz[a]anthracene/Chrysene	0.013	0.014	0.076	0.037
Benzofluoranthenes	0.013	0.016	0.011	0.014
Benzopyrene/Perylene	0.0083	0.012	0.0084	0.0094
Benzo[ghi]perylene	0.0045	0.0099	0.0043	0.0048
Dibenz Phen/Anth[2]	0.0007	0.0014	0.0010	0.00005
Dibenzpyrenes	0.0011	0.0010	0.0007	0.00002

1) Mixture of methylated derivatives of fluoranthene and pyrene.
2) Mixture of dibenz[]phenanthrenes and dibenz[]anthracenes.

C.3.1.2.3 Wood Derived Source Mixtures: Summary

A number of wood-derived source mixtures, including residential fireplaces and wood-burning stoves, as well as open, bush and forest fires, have been examined in sections C.3.1.2.1 and C.3.1.2.2. The data show that the both fuel type and burn conditions can alter the levels of PAH released. Altering either, or both, of these parameters generally does not produce changes in PAH levels by more than ten-fold.

C.3.1.3 Petroleum Derived Source Mixtures

Section C.3.1.3 examines PAH emissions from sources that use petroleum derived products as fuel. Four types of source mixtures are considered: exhaust emissions from diesel and gasoline engines, flue gas emissions from oil-fired power plants and PAH in asphalts. Other source mixtures such as aviation emissions are also derived from petroleum products, however, data for the other sources are extremely limited. Furthermore, the identified mixtures constitute the largest component of emissions generated by petroleum derived fuel.

C.3.1.3.1 Diesel Engine Exhaust Emissions

Exhaust emissions from diesel engines have been shown to be complex mixtures consisting of a large number of chemicals (Schuetzle, 1983; Tong and Karasek, 1984). For analytical purposes, these mixtures are commonly broken down into polar and non-polar fractions. The polar fraction contains large numbers of oxygenated PAH derivatives including ketones, carboxaldehydes, quinones as well as hydroxy and nitro substituted PAH (Schuetzle, 1983; Tong and Karasek, 1984). The non-polar fraction primarily consists of classical and alkylated PAH (Schuetzle, 1983; Tong and Karasek, 1984). Both fractions are frequently divided into smaller sub-fractions. Schuetzle (1983) has provided a partial list of the many compounds contained in each fraction. Tong and Karasek (1984) have identified at least 76 classical and alkylated PAH in the non-polar fraction.

Initial studies of diesel exhaust emissions have focused on the non-polar fraction, as this fraction was believed to be responsible for the tumorigenic activity of the mixtures (Schuetzle, 1983). In addition, the high mutagenic activity of some of the nitro-substituted PAH in the Ames assay has directed attention to the nitro-substituted compounds contained in diesel exhaust (Schuetzle, 1983). Studies which report PAH profiles, seldom report profiles for all polar and non-polar fractions. It is more common to report profiles of either the non-polar or nitro-substituted fractions. Profiles for other fractions are largely unavailable. For these reasons, comparison of mixtures conducted in this appendix deals with the non-polar and nitro-substituted fractions individually. The non-polar and nitro-substituted PAH which have been identified in diesel exhaust emissions are summarized in tables C.3.1.3.1.1 and C.3.1.3.1.2.

Table C.3.1.3.1.1 Classical and alkylated PAH in diesel exhaust emissions.

(all values are rounded to two significant figures)

		PAH Emissions (μg PAH/g extract)				
Compound	**Rings**	**VU**	**VW1**	**VW2**	**VW3**	**Olds**
Acenaphthylene	3	-	30	30	-	-
Phenathrene/Anthracene	3	610	5200[a]	2400[a]	3000[a]	770
Methyl Phen/Anth	3	1400	4300[b]	2500[b]	2900[b]	-
Dimethyl Phen/Anth	3	3200	1300	1800	2400	-
Pyrene	4	1700	8000	3700	3500	450
Fluoranthene	4	1400	7300	3400	3700	540
Methyl Fluoran/Pyr	4	780	2000	1400	1600	-
Chrysene/Triphenylene	4	100	1500	660	870	130
Benz[a]anthracene	4	530	1100	460	500	48
Fluorene	4	-	170	100	170	-
Pyrene, 1-methyl-,	4	-	440	200	140	-
Benz[a]anthracene, methyl-,	4	-	50	30	30	-
Benzo[a]pyrene	5	39	560	270	210	8.0
Benzofluorenes	5	-	1500	760	790	-
Benzo[b]fluoranthene	5	-	1098	420	590	33
Benzo[j]fluoranthene	5	-	1367	490	680	-
Benzo[k]fluoranthene	5	-	289	160	91	-
Benzo[e]pyrene	5	-	950	510	490	-
Dibenz[a,h]anthracene	5	-	96	50	50	-
Indeno[1,2,3-cd]pyrene	5	-	93	30	50	-
Cyclopenta[cd]pyrene	5	17	-	-	-	-
Benzo[ghi]perylene	6	130	420	220	230	-
Dibenzopyrenes	6	-	170	89	90	-
Coronene	7	-	520	300	310	-
Reference		1	2	2	2	3

a) Sum of phenanthrene and anthracene levels.
b) Sum of anthracene, 2-methyl-, phenanthrene, 2-methyl-, and phenanthrene, 3-methyl-.

1) Schuetzle, 1983
2) Tong & Karasek, 1984
3) Eisenberg & Cunningham, 1985

Studies which examined the emissions of nitro-substituted PAH from diesel engines have been reviewed by the International Agency for Research on Cancer (IARC) (WHO, 1989). Table C.3.1.3.1.2 provides a list of nitro-substituted PAH which have been identified in the exhaust of light (LDD) and heavy duty diesels (HDD). For both LDD and HDD, the emissions of dinitro-substituted PAH are lower than those of the mononitro-substituted PAH. The effect of engine speed and load on the release of nitro-substituted PAH from HDD have also been reported (see table C.3.1.3.1.2). Increasing both engine speed and load results in a decrease in the emission of mononitro-substituted PAH and an increase in the emission of dinitro-substituted PAH. The authors suggested that increasing engine speed and/or load increased engine and exhaust temperatures which might enhance the partial oxidation of nitro-substituted PAH and facilitate the addition of further nitro groups to the PAH core (WHO, 1989). The largest changes in the levels of emission are seen with the 3-ring nitro-PAH (see table C.3.1.3.1.2). For these compounds, increasing engine speed and load reduces emissions by one and two orders of magnitude. Changes in the emission levels of 4 and 5-ring nitro-substituted PAH compounds are only one order of magnitude or less. These findings suggest that changes in operating conditions can have a significant effect on the emissions of nitro-substituted PAH.

Table C.3.1.3.1.1 shows that the emission of classical and alkylated PAH varies with the type of engine used and the conditions under which it operates. However, there is little variation among the values. The differences are usually less than two-fold, and only in one situation (pyrene, 1-methyl-,) does the difference exceed three fold. The data for the three Volkswagen (VW) engines were obtained from engines which differed in their driving cycles and fuel and lubrication combinations. The nature and the significance of these differences were not specified by the authors (Tong and Karasek, 1984). This observation suggests that, for a given make of engine, changes in operating conditions may have a limited effect on the levels of PAH emitted.

Emission data for two other diesel engines, an Oldsmobile 350 (Olds) (Eisenberg and Cunningham, 1985) and one of unidentified make (VU) (Schuetzle, 1983) are also listed in table C.3.1.3.1.1. Information on driving cycles and operating conditions were not provided in either report. Therefore, emissions from these engines cannot be compared with each other or with the emissions from the VW engines on a quantitative basis. Qualitatively, the data show differences in the levels of individual PAH among all three engine types. However, it is not possible to determine if these differences are a function of the different diesel engines or if they arise from differences in driving cycles or operating conditions. Albert *et al.* (1983) examined the emissions from a number of different diesel engines operated under similar conditions and found the level of B[a]P varied greatly among engines. This finding suggests that the type of engine does have an effect on the levels of PAH contained in diesel emissions.

Although the data for VW engines suggest that changes in operating conditions, such as driving cycle and lubrication, may not have a significant effect on the levels of classical and alkylated PAH (see table C.3.1.3.1.1), the data for the HDD show that changes in operating conditions (engine speed and load) can have a marked effect on the levels of nitro-substituted PAH emitted (see table C.3.1.3.1.2). In addition, the work of Albert *et al.* (1983) shows that different diesel engines, operated under the same conditions, emit differing levels of B[a]P. Thus, one can conclude that changes in the type of engine and the conditions under which they operate can both effect the levels of PAH in the emissions.

Table C.3.1.3.1.2 Nitro-substituted PAH in diesel exhaust emissions.

(all values are rounded to two significant figures)

	PAH Emissions (µg PAH/g extract)			
	LDD	HDD		
Compound		**Idle**	**High Speed No Load**	**High Speed Full Load**
Anthracene, 9-nitro-,	-	180	35	1.6
Anthracene, 1-methyl-9-nitro-,	-	250	28	1.6
Fluorene, 2-nitro-,	1.2	160	130	15
Fluorene, 2,7-dinitro-,	-	30	39	31
Pyrene, 1-nitro-,	75	28	6.5	1
Pyrene, 1,3-dinidto-,	.030	<1.6	1.3	3.1
Pyrene, 1,6-dinitro-,	0.40	<1.6	2.6	6.3
Pyrene, 1,8-dinitro-,	0.53	<1.6	2.6	6.3
Fluoranthene, 3-nitro-,	3.5	-	-	-
Fluoranthene, 8,-nitro-	1.3	-	-	-
Benzo[a]pyrene, 6-nitro-,	4.2	<6.5	3.5	2.4
Reference	1	2	2	2

1) Paputa-Peck *et al.* 1983
2) WHO, 1989

C.3.1.3.2 Gasoline Engine Exhaust Emissions

Studies which report PAH emissions from a number of gasoline engines are available in the literature (Hoffmann and Wynder, 1962; Alsberg *et al.* 1982; Hagemann *et al.* 1982; Grimmer and Hildebrandt, 1975; Grimmer and Böhnke, 1978). Unfortunately, the data are presented in a number of formats which cannot readily be converted to a common unit. Hoffmann and Wynder (1962), and Grimmer and Böhnke (1978) reported PAH emissions as µg PAH/g of extract; Alsberg *et al.* (1982) and Hagemann *et al.* (1982) reported PAH emissions as µg PAH per distance travelled; Grimmer and Hildebrandt (1975) reported PAH emissions as µg PAH per Europa-Test. As a result, quantitative comparison of total PAH emissions among data sets is not feasible. However, some limited comparison among studies which use the same reporting format is possible. The available data are presented and discussed below.

Hoffmann and Wynder (1962), and Grimmer and Böhnke (1978) have provided emission data for gasoline engines in terms of µg PAH/g extract. The results, summarized in table C.3.1.3.2.1, indicate that the variation in PAH content between the two engine tests is relatively small. In most cases, the two data sets differ by less than three fold. Perylene and benzo[ghi]perylene appear to be the only compounds with larger differences. As the engines and testing protocols differ between the two studies, it would appear that differences in operating conditions have only a small effect on the PAH emissions from gasoline engines. However, this data set is too small to afford any degree of certainty in this observation.

Alsberg *et al.* (1982) and Hagemann *et al.* (1982) have provided data in a µg PAH per distance travelled format. The data are summarized in table C.3.1.3.2.2. Only compounds common to both studies have been included in the table. Alsberg *et al.* (1982) used a US-73 test protocol to monitor PAH emissions from a SAAB 900GL which was not equipped with a catalytic converter. The results from the two tests are summarized in columns 1 and 2 of table C.3.1.3.2.2. Alsberg *et al.* reported the emissions as µg PAH per kilometer travelled. These were converted to µg PAH per mile traveled in table C.3.1.3.2.2. The levels of individual PAH from the two tests (columns 1 and 2) differ by less than three fold for all compounds with the exception of anthanthrene (see table C.3.1.3.2.2). This finding suggests that the emission of individual PAH from a single engine is relatively stable when the engine is run under specific conditions.

Hagemann *et al.* (1982) used a different test protocol, the US-FTP 75 test protocol, to monitor PAH emissions from three engines of an unspecified type, all of which were equipped with catalytic converters. The results are summarized in columns 3, 4 and 5 of table C.3.1.3.2.2. The levels of individual PAH vary little among the three engines tested. The difference between the highest and the lowest levels is less than three fold for the majority of the compounds tested.

The largest difference was reported for perylene (a seven-fold difference between the lowest and the highest level). These data suggest that the emission of individual PAH varies little among gasoline engines when the engines are run under the same operating conditions.

Table C.3.1.3.2.1 PAH emission from gasoline engine exhaust (µg PAH/g extract).

(all values are rounded to two significant figures)

Compound	µg PAH/g Extract	
	Hoffmann & Wynder, 1962	Grimmer *et al.* 1975, 1978
Fluoranthene	970	2000
Fluoranthene, Alkylated	260	-
Benzo[a]pyrene	32	260
Benzo[a]pyrene, Alkylated	1.0	-
Dibenz[a,h]anthracene	2.3	-
Benzo[b]fluoranthene	64	150
Benzo[j]fluoranthene	17	87
Benzo[k]fluoranthene	54	58
Benzo[mno]fluoranthene	150	-
Benz[a]anthracene	62	91
Benzo[e]pyrene	420	200
Chrysene	180	180
Chrysene, Alkylated	67	-
Benz[a]anthracene, Alkylated	2.0	-
Indeno[1,2,3-cd]fluoranthene	2.6	-
Indeno[1,2,3-cd]pyrene	82	250
Anthanthrene	44	-
11-H-Benzo[b]fluorene	51	-
Perylene	-	55
Benzo[ghi]perylene	51	770
Triphenylene	44	91
Pyrene	1800	37
Pyrene, Alkylated	8.0	-
Coronene	340	390

Table C.3.1.3.2.2 PAH emission from gasoline engine exhaust (µg PAH/mile).

(all values are rounded to two significant figures)

	PAH Emissions (µg PAH/mile)				
Compound	Column 1	Column 2	Column 3	Column 4	Column 5
Fluoranthene	32	23	0.09	0.13	0.22
Pyrene	45	50	0.18	0.20	0.37
Chrysene	11	14	0.88	0.17	0.26
Benzofluor & Methyl-Pyr[1]	42	45	0.11	0.06	0.10
Benzo[ghi]fluoranthene	9.0	19	0.15	0.14	0.25
Benzo[b,j&k]fluoranthenes	8.1	13	0.53	0.55	0.90
Benzo[e]pyrene	4.2	10	0.88	0.72	1.5
Benzo[a]pyrene	3.1	7.2	0.70	0.72	1.9
Perylene	0.48	0.80	0.22	0.33	1.6
Indeno[1,2,3-cd]pyrene	2.7	5.8	1.4	1.0	2.1
Benzo[ghi]perylene	9.5	21	2.2	1.3	2.2
Anthanthrene	0.32	2.1	0.22	0.15	.015
Coronene	10.5	19	3.5	1.2	1.8
Reference	1	1	2	2	2

1) Alsberg *et al.* 1982
2) Hagemann *et al.* 1982

There are large differences of up to almost three orders of magnitude in PAH emissions between the work of Alsberg *et al.* (1982) and Hagemann *et al.* (1982). These differences may be attributed, in part, to differences in the test protocols used in the studies. However, it is more likely that the observed differences result from the presence or absence of a catalytic converter in the exhaust train of the test vehicles. Alsberg *et al.* (1982) used a vehicle without a catalytic converter while the vehicles used by Hagemann *et al.* (1982) were equipped with new catalytic converters. Hagemann *et al.* (1982) studied the effect of the age of the catalytic converter on the levels of PAH emissions and found the emission levels from "old" or used converters were up to forty fold greater than those from a new converter (data not shown).

Table C.3.1.3.2.3a PAH emission from gasoline engine exhaust (µg PAH/Europa-test) Models 1-10.

(all values are rounded to two significant figures)

	PAH Emissions (µg PAH/Europa-test)									
Compound	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**
Phenanthrene	1200	690	1000	630	710	600	790	520	1300	1300
Anthracene	370	220	260	88	210	160	250	140	370	290
Phenanthrene, 3-methyl-,	440	280	390	310	370	3330	420	250	430	610
Phenanthrene, 2-methyl-,	470	300	400	330	420	360	460	240	450	640
Fluoranthene	490	280	380	250	460	270	490	310	460	530
Pyrene	610	420	420	320	670	370	670	400	530	630
Chrysene	270	56	170	62	130	54	150	81	200	180
Benzo[b,j&k]fluoranthenes	52	18	34	19	34	13	32	20	100	68
Benzo[e]pyrene	45	19	28	18	30	12	33	21	65	49
Benzo[a]pyrene	39	18	23	17	24	10	27	18	68	46
Indeno[1,2,3-cd]pyrene	37	13	20	15	24	8.3	21	12	71	28
Benzo[ghi]perylene	150	60	86	69	120	38	52	38	220	120
Anthanthrene	34	8.9	6.5	9.8	18	4.9	5.9	4.7	40	18
Coronene	87	45	27	60	86	28	52	26	150	64

Table C.3.1.3.2.3b PAH emission from gasoline engine exhaust (µg PAH/Europa-test) Models 11-20.

(all values are rounded to two significant figures)

	PAH Emissions (µg PAH/Europa-test)									
Compound	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20
Phenanthrene	450	720	790	920	2500	930	760	630	590	880
Anthracene	130	120	140	220	600	260	210	170	150	250
Phenanthrene, 3-methyl-,	230	440	350	460	790	440	320	290	340	380
Phenanthrene, 2-methyl-,	260	470	360	480	860	480	350	320	370	450
Fluoranthene	290	550	360	590	1200	320	380	280	240	440
Pyrene	380	880	460	930	1700	350	450	410	340	730
Chrysene	100	290	64	130	620	72	280	97	60	340
Benzo[b,j&k]fluoranthenes	24	53	24	42	120	23	40	20	15	50
Benzo[e]pyrene	20	55	23	40	92	22	36	18	13	42
Benzo[a]pyrene	19	58	19	45	120	17	37	14	12	51
Indeno[1,2,3-cd]pyrene	20	45	17	33	99	10	26	10	9.3	40
Benzo[ghi]perylene	81	180	66	130	300	44	150	50	39	150
Anthanthrene	15	33	7.9	24	84	3.6	32	7.8	5.1	48
Coronene	65	110	34	78	160	17	98	34	32	80

The combined findings of Alsberg *et al.* (1982) and Hagemann *et al.* (1982) suggest that, within a given set of operating conditions, there is little variation in the emission levels of individual PAH. On the other hand, changes in operating conditions, such as aging of the catalytic converter, or its removal from the exhaust train, can have a significant effect on the emission of PAH. Hence, consideration should be given to the type of emission controls in use when evaluating PAH emissions from gasoline engines. It should be noted, however, that both of these data sets are relatively small. Therefore, these conclusions must be viewed with caution.

Grimmer and Hildebrandt (1975) monitored PAH emissions from twenty different and unspecified car models. Five cars from each model were tested and a standard fuel was used in all cars. The authors had not reported if a catalytic converter was present in each model. However, as all cars tested were registered in West Germany between 1971 and 1975 (date of publication), it is unlikely that any were equipped with catalytic converters in the exhaust train. The PAH emission data summarized in tables C.3.1.3.2.3a and C.3.1.3.2.3b are the geometric mean values for four cars from each model. The four chosen cars had been tested using the same protocol. The fifth car in each group was tested using a different driving cycle and was therefore excluded from the analysis. Differences between the lowest and the highest reported levels for individual PAH ranged between three and 23-fold for the 20 models tested. Model 15 "M15" showed the highest emission values for all PAH tested. No one car consistently showed the lowest values. No explanation has been provided for the high emission values for M15 in the report.

The data presented in tables C.3.1.3.2.2, C.3.1.3.2.3a and b show that different engines, operated under set conditions (same driving cycle, fuel, emission controls, etc), emit PAH at levels which generally vary by less than ten-fold. This characteristic can be illustrated by the work of Hagemann *et al.* (1982) (see table C.3.1.3.2.2, columns 3, 4 and 5), where differences in emissions among engines were less than three-fold for eleven of the thirteen PAH, and less than ten-fold for all compounds tested The work of Grimmer and Hildebrandt (1975) shows the same result with a larger data set involving 76 vehicles (see table C.3.1.3.2.3a and b). The differences in emissions of individual PAH from 19 of the 20 vehicle models tested were less than ten-fold. The size of the data set (reported by Grimmer and Hildebrandt) provides a reasonable degree of confidence for the observation. The information on fuel type is insufficient to adequately evaluate if the type of fuel used has any effect on PAH emission levels for gasoline engines.

C.3.1.3.3 Oil Fired Power Plants

Bennet *et al.* (1979) monitored PAH emissions from a single oil-fired power plant on three separate visits. Three samples were taken on the first visit and only single samples were taken on the two subsequent visits. Different fuels were burned at each visit and the power output

also varied. The results of the sampling are summarized in table C.3.1.3.3.1. The greatest variation in levels is seen for methyl anthracenes, with a 2100 fold difference between the highest and the lowest reported values. It should be noted that this difference arose from two samples taken on the first visit, when power output and fuel should not have changed. The reason for the difference was not addressed by the authors. A similar difference is observed for anthracene/phenanthrene with a 250 fold difference reported between samples 1 and 3 from the first visit. Because of these unexplained variations, the geometric means of the samples from the first visit have been used to compare with the values reported from the other two visits. Comparing the mean values from visit 1 with the other data shows that the levels of the larger molecular weight PAH (≥ four rings) vary between one and two orders of magnitude (see table C.3.1.3.3.1). These data suggest that changes in fuel type or power output can have a significant effect on the levels of PAH emitted. Because the authors did not indicate what the burn conditions were for each sample, it is not possible to determine the contribution of either fuel type or power output to the overall observed changes.

C.3.1.3.4 PAH in Petroleum Derived Asphalts

Bitumens (asphalts) occur naturally and can also be derived during the petroleum refining process. Naturally occurring bitumens include natural asphalts, tar sands, rock asphalts and lake asphalts (WHO, 1985). Fractional distillation of crude petroleum leaves a heavy residue called bitumens or straight run asphalt (WHO, 1985, Malaiyandi *et al.* 1982). This section will focus only on bitumens that are derived from petroleum.

Eight classes of bitumens have been identified by IARC (WHO, 1985) and represent the major types of bitumens used in industry. The terms "bitumens" and "asphalts" are used interchangeably in the literature. The term "asphalts" will be used exclusively in appendix C, as this term is used more commonly in North America than the term "bitumens". A detailed description of the eight classes can be found in the IARC monograph on bitumens (WHO, 1985). While eight classes of bitumens have been identified, PAH profiles appear to be available for only two, Class 1 (*Penetration Asphalts*) and Class 2 (*Oxidized Asphalts*). Therefore, appendix C discusses only Classes 1 and 2 asphalts. Penetrating Asphalts are typically used for road paving, while Oxidized Asphalts are used in roofing applications. Therefore the two classes of asphalts will be described as *paving* and *roofing* asphalts, respectively.

Table C.3.1.3.3.1 PAH emissions in flue-gases from oil-fired power plants.

(all values are rounded to two significant figures)

	PAH Emissions (μg PAH/m³)					
	Visit #1				Visit #2	Visit #3
Compound	S1	S2	S3	Mean	S1	S1
Anthracene/Phenanthrene	2.1	0.011	0.0083	0.058	0.089	0.21
Anthracene, methyl-,	0.21	<0.0002	<0.0001	0.0020	0.014	0.033
Fluoranthene	0.098	0.010	0.0065	0.019	0.018	0.052
Pyrene	0.051	0.0099	0.0063	0.015	0.022	0.026
Methyl Fluoran/Pyr	0.056	0.0043	<0.0001	0.0030	<0.0001	<0.0012
Benzo[c]phenathrene	<0.0001	<0.0001	<0.0001	<0.0001	0.0030	<0.0012
Benz[a]anthracene/Chrysene	<0.0001	<0.0001	<0.0001	<0.0001	0.0039	<0.0012
Chrysene, methyl-,	<0.0001	<0.0001	<0.0001	<0.0001	0.0044	<0.0012
Benz[a]anthracene, 7,12-dimethyl-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0012
Benzo[b,j&k]fluoranthenes	<0.0001	<0.0001	<0.0001	<0.0001	0.0093	<0.0012
Benzo[a]pyrene	<0.0001	<0.0001	<0.0001	<0.0001	0.0024	<0.0012
Benzo[e]pyrene	<0.0001	<0.0001	<0.0001	<0.0001	0.0057	<0.0012
Perylene	<0.0001	<0.0001	<0.0001	<0.0001	0.00080	<0.0012
Benzopyrenes, methyl-,	<0.0001	<0.0001	<0.0001	<0.0001	0.0031	<0.0012
Cholanthrene, 3-methyl-,	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0012
Indeno[1,2,3-cd]pyrene	<0.0001	<0.0001	<0.0001	<0.0001	0.00021	<0.0012
Benzo[ghi]perylene	<0.0001	<0.0001	<0.0001	<0.0001	0.00047	<0.0012
Dibenz[a,h]anthracene	<0.0001	<0.0001	<0.0001	<0.0001	0.00059	<0.0012
Dibenz[a,i & a,h]pyrenes	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0012
Coronene	<0.0001	<0.0001	<0.0001	<0.0001	0.00030	<0.0013

The PAH contents of a number of paving asphalts have been reported by IARC (WHO, 1985) and Malaiyandi *et al.* (1982), and are summarized in table C.3.1.3.4.1. Columns A through G report PAH levels in 7 unidentified paving asphalt samples (WHO, 1985). Columns I through L show values for four penetrating asphalts, where the values 50/60 and 80/100 identify the penetration grade (WHO, 1985). The final columns (PA #1 and PA #2) show PAH levels in

asphalt samples reported by Malaiyandi *et al.* (1982). The levels of individual PAH vary by as much as three orders of magnitude across all reported samples. The lowest and highest reported values vary by more than two orders of magnitude for four of the ten compounds listed (pyrene, benz[a]anthracene, perylene and benzo[a]pyrene) and by 2100 fold for chrysene. Only two compounds (7,12-dimethylbenz[a]anthracene and dibenz[a,h]anthracene) show a difference of less than ten fold between the highest and the lowest levels. These two compounds have only been reported by Malaiyandi *et al.* (1982).

Direct comparison among all samples must be done with care as the grade of asphalt is not provided for all samples. In cases where the grade is provided (3 samples of 80/100), the PAH content does not vary greatly among samples (see table C.3.1.3.4.1). For all but one of the compounds (perylene), the differences between the lowest and highest levels are less than three-fold. For perylene, the difference is approximately 13-fold. For the paving asphalt samples reported by Malaiyandi *et al.* (1982), differences in PAH levels are less than two-fold. The data show that within a grade of paving asphalt, the differences in the levels of individual PAH are small. However, there appears to be a considerable degree of variation in the levels of individual PAH among paving asphalts of different grades. Some of the differences can be as large as three orders of magnitude.

Table C.3.1.3.4.2 reports PAH levels for three grades of oxidized asphalts (85/40, 110/30 and 95/25) and for three oxidized asphalts, specifically identified as roofing asphalts (RA#1, RA#2 and RA#3) (Malaiyandi *et al.* 1982). For the purpose of the present assessment, all will be considered as roofing asphalts. Within the three products identified as 85/40, 110/30 and 95/25, the levels of individual PAH vary by no more than three-fold. However, a greater degree of variation in the levels of individual PAH is evident among the remaining three samples, RA#1, RA#2 and RA#3. Further, on a μg/g basis, the levels of PAH in RA#1, RA#2 and RA#3 are more than two orders of magnitude higher than those reported for the other three roofing asphalts. These data suggest that a large variation in PAH content exists among roofing asphalts.

C.3.1.3.5 Oil-Derived Source Mixtures: Summary

The PAH emissions from a number of oil-derived source mixtures have been examined in sections C.3.1.3.1 through C.3.1.3.3. In addition, the PAH content of some asphalts have been reviewed in section C.3.1.3.4. Direct comparison among many of these source mixtures is not possible as different, and non-convertible reporting formats have been used by different authors to describe the different mixtures. The data presented for diesel and gasoline engine exhaust emissions and flue-gas emissions from oil-fired power plants clearly show that operating conditions can have a marked effect on PAH emission levels. The diesel and gasoline engine data also show that differences in PAH emissions are small among engines running under similar conditions. In contrast, the PAH content of different asphalts can vary by a wide margin.

APPENDIX E

STANDARDIZED NOMENCLATURE FOR POLYNUCLEAR AROMATIC HYDROCARBONS

by Bryan Leece

Table C.3.1.3.4.1 PAH content of paving asphalt (Malaiyandi *et al.* 1982).

(all values are rounded to two significant figures)

	PAH Content (μg PAH/g Asphalt)												
Compound	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**I (50/60)**	**J (80/100)**	**K (80/100)**	**L (80/100)**	**PA#1**	**PA#2**
Fluoranthene	-	-	2.0	-	-	5.0	-	0.41	0.72	0.46	0.39	3.3	2.7
Pyrene	0.6	1.8	4.0	8.3	0.9	38	0.3	0.26	1.5	1.0	1.1	10	9.5
Benz[a]anthracene	0.25	2.1	1.1	0.7	0.9	35	0.2	0.14	1.1	0.89	0.63	100	84
Chrysene	0.2	8.9	2.3	3.9	3.2	34	0.7	0.47	1.5	3.3	3.9	85	79
Benz[a]anthracene, 7,12-diemthyl-,	-	-	-	-	-	-	-	-	-	-	-	4.8	4.1
Perylene	-	39	2.2	6.1	2.9	3.0	0.10	0.044	3.3	0.69	0.25	1.7	1.4
Benzo[k]fluoranthene	-	-	-	-	-	-	-	0.024	0.19	-	-	2.0	1.8
Benzo[a]pyrene	0.5	1.7	1.3	2.5	1.6	27	0.10	0.22	1.8	0.92	1.1	1.3	1.3
Benzo[ghi]perylene	2.1	4.6	1.0	1.7	2.7	15	06	1.7	4.2	2.3	2.7	1.6	0.92
Dibenz[a,h]anthracene	-	-	-	-	-	-	-	-	-	-	-	3.7	5.8

Table C.3.1.3.4.2 PAH content of roofing asphalt (Malaiyandi *et al.* 1982).

(all values are rounded to two significant figures)

	PAH Content (µg PAH/g Asphalt)					
Compound	**85/40**	**110/30**	**95/25**	**RA#1**	**RA#2**	**RA#3**
Fluoranthene	0.15	0.4	0.46	13	15	5.2
Pyrene	0.17	0.3	0.29	-	32	5.5
Benz[a]anthracene	0.33	0.30	0.23	380	170	110
Chrysene	0.90	1.0	0.80	380	120	45
Benz[a]anthracene, 7,12-diemthyl-,	-	-	-	9.0	11	0.71
Perylene	0.14	0.08	0.20	10	3.5	2.3
Benzo[k]fluoranthene	0.051	0.10	0.04	2.8	6.1	0.29
Benzo[a]pyrene	0.49	0.35	0.48	8.1	5.4	1.8
Benzo[ghi]perylene	1.3	1.2	2.0	5.5	4.2	4.3
Dibenz[a,h]anthracene	-	-	-	6.7	-	0.39

In general, difference in operating conditions appears to have the largest effect on PAH emissions from oil-derived source mixtures. However, the available data are insufficient to adequately evaluate the effect of fuel-types on PAH emissions.

C.3.1.4 Other Source Mixtures

The only other source mixture which has been examined is cigarette smoke condensate. The PAH levels in this mixture are described in section C.3.1.4.1.

C.3.1.4.1 Cigarette Smoke Condensate

The available data on PAH levels in cigarette smoke condensate have been extensively reviewed by IARC (WHO, 1986) and summarized in table C.3.1.4.1.1. Both mainstream smoke (MSS) and sidestream smoke (SSS) are described. Where more than one report are available, table C.3.1.4.1.1 follows IARC's practice and presents the data as ranges.

In MSS, for PAH for which ranges have been reported, the differences between the lowest and highest reported levels of individual PAH range from two-fold for benzo[k]fluoranthene, benzo[rst]pentaphene and perylene, to fifty-fold for indeno[1,2,3-cd]pyrene. Of the eighteen compounds for which ranges are available, eight vary by more than ten-fold in levels (see table C.3.1.4.1.1). IARC (WHO, 1986) indicates that the type of tobacco and smoking rate both have an effect on the levels of PAH produced but does not address the contribution that each of these factors makes to the overall variation in PAH levels.

The data on SSS provided by IARC are limited to only a few PAH. Of these compounds, range data are available only for pyrene and benzo[a]pyrene, whose levels vary by less than ten-fold. Otherwise, the data are too limited to permit adequate evaluation of the variations in PAH levels in SSS. Nevertheless, the dependence of PAH emissions on the type of tobacco and the smoking rate for MSS likely holds for SSS as well.

PAH levels are generally higher in SSS than in MSS (see C.3.1.4.1.1). The differences in levels range between 1.8 fold for the high range value of anthanthrene in MSS and the level of anthanthrene in SSS to 130-fold for the low value of fluoranthene reported in MSS and the level reported in SSS. One of the major differences between SSS and MSS is the temperature at which they are formed. During active inhalation, MSS is formed at about 800°C to 900°C. Between periods of inhalation, SSS is formed at temperatures around 600°C (WHO, 1986). The difference in the combustion condition appears to have an effect on the PAH content of the mixtures. However, the available data on the type of tobacco or smoking rate for either MSS or SSS do not allow for further quantitative comparison. Even though both mixtures contribute to overall human exposure, the data indicate that MSS and SSS should not be considered equivalent mixtures.

Table C.3.1.4.1.1 PAH levels in cigarette smoke condensate (WHO, 1986).

(all values are rounded to two significant figures)

		µg PAH/100 Cigarettes	
Compound	**Rings**	**MSS**	**SSS**
Anthracene	3	2.3-24	-
Phenanthrene	3	8.5-62	-
Phenanthrene, 1-methyl-,	3	3.2	-
Benz[a]anthracene	4	0.40-7.6	-
Chrysene	4	0.60-9.6	-
Chrysene, 1-methyl-,	4	0.30	-
Chrysene, 2-methyl-,	4	0.12	-
Chrysene, 3-methyl-,	4	0.61	-
Chrysene, 5-methyl-,	4	0.060	-
Chrysene, 6-methyl-,	4	0.70	-
Fluoranthene	4	1.0-27	130
1H-Benzo[a]fluorene	4	4.1-18	75
1H-Benzo[b]fluorene	4	2.0	-
Pyrene	4	5.0-27	39-100
Benzo[b]fluoranthene	5	0.40-2.2	-
Benzo[j]fluoranthene	5	0.60-2.1	-
Benzo[k]fluoranthene	5	0.60-1.2	-
Benzo[ghi]fluoranthene	5	0.10-0.40	-
Benzo[a]pyrene	5	0.50-7.8	2.5-20
Benzo[e]pyrene	5	0.20-2.5	14
Dibenz[a,h]anthracene	5	0.40	-
Dibenz[a,j]anthracene	5	1.1	4.1
Indeno[1,2,3-cd]pyrene	5	0.40-20	-
Perylene	5	0.30-0.56	3.9
Anthanthrene	6	0.20-2.2	3.9
Benzo[ghi]perylene	6	0.30-3.9	9.8
Dibenzo[a,i]pyrene	6	0.17-0.32	-
Coronene	7	0.10	-

C.3.1.5 Comparison of Source Mixtures

The PAH content of a number of individual source mixtures have been examined in sections C.3.1.1 through C.3.1.4. However, they cannot be directly compared because the levels from different studies have been reported in incompatible formats (see table C.3.1.5.1). The data are summarized in tables C.3.1.5.2 through C.3.1.5.5.

Table C.3.1.5.1 PAH source mixture: data formats.

Format	Source Mixture	Section
µg PAH/m^3 Air Sampled	Coke Oven Emissions	3.1.1.1
	Iron and Steel Foundries	3.1.1.3
	Coal-Fired Power Plants	3.1.1.4
	Residential Coal Stoves	3.1.1.5
	Residential Wood Stoves	3.1.2.2
	Oil-Fired Power Plants	3.1.3.3
	Asphalt	3.1.3.4
mg PAH/kg Fuel	Residential Coal Stoves	3.1.15
	Residential Wood Stoves	3.1.2.2
mg PAH/g Starting Material	Low Temperature Coal Tars	3.1.1.2.1
	High Temperature Coal Tars	3.1.1.2.2
	Asphalt	3.1.3.4
µg PAH/g Particulate	Open Wood Burning	3.1.2.1
	Forest Fires	3.1.2.1
µg PAH/g Particulate Extract	Diesel Engine Exhaust	3.1.3.1
	Gasoline Engine Exhaust	3.1.3.2

The most common reporting unit for PAH levels is µg PAH/m^3 air sampled. Table C.3.1.5.2 summarizes levels of individual PAH from source mixtures reported using this format. The table provides both the highest and the lowest values, as well as the range for each PAH. The data show that levels can differ by over six orders of magnitude for some PAH. This finding suggests that differences in both fuel and operating conditions can affect the levels of PAH emitted. Differences in the sampling technique may also account for some of the observed differences. For example, a sampler placed immediately adjacent to a source could reasonably be expected to detect higher levels of PAH than a sampler removed from the source. Therefore, comparing emission levels among sources must be done with caution. Some comparison can however, be made.

Table C.3.1.5.2 PAH emissions from various sources (μg PAH/m^3 air sampled format).

(all values are rounded to two significant figures)

		Compound													
Source	Sample	Anth	Phen	An/Ph	Fluor	Pyr	B[a]A	Ch/Tr	Pery	BFlo	B[a]P	B[e]P	BghiP	DBA	Cor
Coke Oven	Spring	35	12	47	-	.05	-	-	-	-	-	-	-	-	-
	Fall	119	31	150	9.5	4.9	0.8	0.9	-	-	0.9	0.4	-	-	-
Foundry		2.3	4.5	6.8	1.6	1.7	0.67	0.82	0.21	0.87	0.47	0.48	0.72	0.24	0.21
Coal Power	Plt A	0.04	0.37	0.41	0.80	.0.31	0.08	0.37	-	0.23	0.07	0.8	-	-	-
	Plt B	0.05	0.85	0.90	0.38	0.18	0.04	0.19	0.01	0.1	-	0.1	0.1	-	-
	Plt C	0.10	0.10	0.20	0.10	0.10	0.10	0.10	0.10	-	0.10	0.10	0.10	-	-
	Plt D	0.10	0.10	0.20	0.10	0.10	0.10	0.10	0.10	-	0.10	0.010	0.010	-	-
	Plt E	-	0.10	0.10	0.010	0.070	-	0.010	0.010	-	0.010	0.010	0.010	-	-
	Plt F	0.10	0.29	0.29	0.10	0.10	0.10	0.10	0.10	-	0.10	0.10	0.10	-	-
	Plt G	0.10	15.3	15.3	22.1	0.91	1.4	0.10	0.10	0.88	0.79	0.81	0.10	-	-
	Plt H	109	657	766	227	205	32.2	18.4	2.1	26	12.5	12.6	3.9	1.0	-
	Plt I	0.9	16.7	17.6	5.7	2.9	0.10	0.10	0.10	-	0.10	0.10	0.10	-	-
	Plt J	-	1.0	1.0	1.0	1.0	0.60	0.60	-	-	0.20	-	-	-	-
	U.S. P1	-	-	0.018	0.0019	0.0004	-	-	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	U.S. P2	-	-	0.24	0.015	0.011	-	-	0.0008	0.0002	0.0002	-	0.0002	0.0002	0.0002
	U.S. P3	-	-	0.22	0.060	0.032	-	-	0.0022	0.0003	0.0003	-	0.0002	0.0002	0.0002
Coal Stoves	A. B.	-	-	-	380	87	160	-	140	227	65	200	96	23	41
	L. B.	-	-	-	70	15	21	-	13	18.2	5.0	34	20	10	6.3
	Anth	-	-	-	0.087	0.025	0.069	-	0.023	0.19	0.012	0.19	0.027	0.013	0.0076
	B. C.	-	-	-	-	0.0017	0.0087	-	0.017	0.014	0.010	1.2	0.12	-	0.038
	Anzit	-	-	-	3.9	0.13	1.8	-	0.014	0.19	0.0022	0.68	0.023	0.054	0.011
	Ezit	-	-	-	0.028	0.0044	0.0045	-	-	0.017	0.0005	0.032	0.0051	-	0.0016

Table C.3.1.5.2 PAH emissions from various sources (μg PAH/m^3 air sampled format).....continued.

		Compound													
Source	**Sample**	**Anth**	**Phen**	**An/Ph**	**Fluor**	**Pyr**	**B[a]A**	**Ch/Tr**	**Pery**	**BFlo**	**B[a]P**	**B[e]P**	**BghiP**	**DBA**	**Cor**
Wood Stoves	Bch N	87	620	707	300	290	51	65	29	-	60	37	58	-	-
	Bch AS	95	290	385	76	85	19	27	1.4	-	14	9.5	11	-	-
	Spr N	14	96	110	34	27	6.9	-	2.6	-	5.3	4.0	3.9	-	-
	Spr AS	170	780	950	300	350	72	77	3.5	-	57	32	26	-	-
Oil Power	V1 M	-	-	0.058	0.019	0.015	-	-	0.001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	V2	-	-	0.089	0.018	0.022	-	-	0.0008	0.093	0.0024	0.0057	0.0005	0.0006	0.0003
	V3	-	-	0.21	0.052	0.026	-	-	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012	0.0013
Asphalt	PA #1	-	-	-	1.2	0.51	0.23	-	0.01	-	-	-	0.04	0.01	-
	PA #2	-	-	-	0.44	0.04	0.03	-	0.01	-	-	-	0.03	-	-
	RA #1	-	-	-	0.53	0.10	0.11	-	-	-	0.01	-	0.04	-	-
	RA #2	-	-	-	0.22	0.21	0.17	-	0.01	-	0.01	-	0.10	-	-
	RA #3	-	-	-	0.09	0.05	0.14	-	0.02	-	-	-	0.03	0.01	-
Lowest		0.04	0.10	0.018	0.018	0.0004	0.0045	0.010	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Highest		170	780	950	380	350	160	77	140	227	65	200	96	23	41
Range		4250	7800	52777	21111	8.8E5	35555	7700	1.4E6	2.3E6	6.5E5	2.0E6	9.6E5	2.3E5	4.1E5

Plt A = European Power Plant A-J
A.B. = Anthracite Briquettes
L.B. = Lignite Briquettes
B.C. = Broken Coke
Ezit = Extrazit

Anth = Anthracene
Phen = Phenanthrene
An/Ph = Anthracene/Phenanthrene
Fluor = Fluoranthene
Pyr = Pyrene

Bch N = Birch Normal
Bch AS = Birch Air Starved
Spr N = Spruce Normal
Spr AS = Spruce Air Starved
V1 M = Visit #1 Mean

B[a]A = Benz[a]anthracene
Ch/Tr = Chrysene/Triphenylene
Pery = Perylene
BFlo = Benzofluoranthenes
B[a]P = Benzo[a]pyrene

V2 = Visit #2
V3 = Visit #3
PA #1-2 = Paving Asphalt #1-2
RA #1-3 = Roofing Asphalt #1-3

B[e]P = Benzo[e]pyrene
BghiP = Benzo[ghi]perylene
DBA = Dibenz[a,h]anthracene
Cor = Coronene

Comparing PAH emissions between coal and oil-fired power plants shows that the emissions from oil-fired plants located in the United States (V1M, V2 and V3 in table C.3.1.5.2) are similar to those from U.S. coal-fired plants (U.S.P1, U.S.P2, U.S.P3 in table C.3.1.5.2). Emissions from coal-fired plants in Europe (Plt A through J in table C.3.1.5.2) are generally higher than those from either type of plant in the U.S. However, the cited publications have not provided sufficient information to allow the determination of whether these differences are due to differences in the fuel or operating conditions or to a difference in the sampling technique.

Table C.3.1.5.3 PAH emissions from various sources: mg PAH/kg fuel burned format.

(all values are rounded to two significant figures)

	PAH Emissions (mg PAH/kg Fuel Burned)					
	Coal Stoves		Wood Stoves			
Compound	SCS[1]	PCS[2]	WS1[3]	WS2[4]	WS3[5]	WS4[6]
Fluoranthene	3.3	15	0.018	0.032	0.021	0.019
Pyrene	3.0	12	0.016	0.024	0.017	0.019
Benz[a]anthracene	1.1	3.5	-	-	-	-
Chrysene	1.6	5.4	-	-	-	-
Benzofluoranthenes	0.95	3.0	0.013	0.016	0.011	0.014
Benzo[a]pyrene	0.46	1.2	-	-	-	-
Benzo[e]pyrene	0.46	1.6	-	-	-	-
Perylene	0.20	0.44	-	-	-	-
Indeno[1,2,3-cd]pyrene	0.21	0.55	-	-	-	-
Benzo[ghi]perylene	0.035	0.50	0.0045	0.0099	0.0043	0.0048

1) Slow combustion coal stove
2) Permanent combustion coal stove
3) Baffled wood stove (seasoned oak)
4) Baffled wood stove (seasoned pine)
5) Non-baffled stove (seasoned oak)
6) Non-baffled stove (green pine)

Table C.3.1.5.4 PAH emissions from various sources: mg PAH/g starting material format.

(all values are rounded to two significant figures)

	mg PAH/g Starting Material								
	Coal Tars				Asphalts				
Compound	**LTCT**	**HTCT1**	**HTCT2**	**HTCT3**	**PA1**	**PA2**	**RA1**	**RA2**	**RA3**
Fluoranthene	0.18	33	30	40	0.0033	0.0027	0.013	0.015	0.0052
Pyrene	0.11	21	99	31	0.010	0.0095	-	0.032	0.0055
Benz[a]anthracene	0.14	6.5	-	8.0	0.10	0.084	0.38	0.17	0.11
Chrysene/Triphenylene	0.090	11	24	8.6	-	-	-	-	-
Perylene	0.11	2.5	5.5	4.2	0.0017	0.0014	0.010	0.0035	0.0023
Benzo[a]pyrene	0.080	5.5	4.0	9.7	0.0013	0.0013	0.081	0.0054	0.0018
Benzo[ghi]perylene	-	5.5	-	7.0	0.0016	0.00092	0.0055	0.0042	0.0043
Dibenz[a,h]anthracene	-	1.0	-	-	0.0037	0.0058	0.0067	-	-
Benzo[b,j&k]fluoranthenes	0.14	10	-	11	-	-	-	-	-
Indeno[1,2,3-cd]pyrene]	-	5.0	-	8.0	-	-	-	-	-

PA = Paving asphalt
RA = Roofing asphalt

The levels of PAH emitted from wood-burning stoves are similar to those from residential coal stoves burning either lignite or anthracite briquettes, and are substantially higher than form stoves burning other coal fuels (see table C.3.1.5.2). PAH emissions from wood stoves are one to two orders of magnitude lower than those from coal stoves when compared on a mg PAH/kg fuel burned basis (see table C.3.1.5.3). It should be noted that the burning of wood and coal burning used different types of stove in the studies. Therefore, it is difficult to attribute differences in emission levels to fuel type alone. Differences in the operation of the stoves and also in sampling techniques may contribute to the reported differences in PAH emission levels. To adequately address the differences between wood and coal burning, it is necessary to compare the fuels burned under the same conditions. This type of data is not available for the present analysis.

Table C.3.1.5.4 presents a summary of the PAH emissions from source mixtures whose PAH contents are reported as mg PAH/g starting material. The data indicate that PAH levels are higher in the HTCT than in either the LTCT or the roofing or paving asphalts. With the present information, it is difficult to determine if these differences are due to differences in the starting material (both LTCT and HTCT are derived from coal, while asphalts are petroleum based), or differences in the production processes. As discussed in section C.3.1.1.2, the temperature of formation has a marked effect on the PAH formed for both LTCT and HTCT. Since asphalts are formed at temperatures below 600°C while HTCT are formed at temperature greater than 700°C (see section C.3.1.1.2), it is likely that the differences in PAH levels between HTCT and asphalts are largely a function of the difference in the temperature of formation.

Emissions from open wood burning and forest fires are the only data sets which report emissions on a µg PAH/g particulate basis. A comparison of these two data sets can be found in section C.3.1.2.1.

Only limited data on exhaust emissions from diesel and gasoline engines report PAH levels using the µg PAH/g particulate extract format. The data are summarized in table C.3.1.5.5. The only gasoline engine data which could be included are those of Hoffmann and Wynder (1962) and Grimmer *et al.* (1978). The other gasoline engine PAH emission data are presented in formats which are not comparable with these studies (see Section 3.1.3.2). The data indicate that, for five of the eight compounds listed, the levels in diesel and gasoline engines emissions are similar. For the remaining three compounds, the levels in gasoline engine exhausts appear to be lower than those found in diesel exhaust. It is difficult to determine if these observed differences are due to fuel type or operating conditions.

In summary, comparison of PAH levels among mixtures is hampered by the use of multiple reporting formats. Where the same formats are used and comparisons can be made, PAH levels are found to vary by up to six orders of magnitude (see table C.3.1.5.2). The data also suggest that changes in both the fuel type and operating conditions can have an effect on the PAH levels in various

source emissions. In addition, differences in sampling techniques also affect the levels of PAH reported. However, it is not possible to determine what individual contribution each of these factors actually makes to the overall reported differences.

Table C.3.1.5.5 PAH emissions from various sources: µg PAH/g particulate extract.

(all values are rounded to two significant figures)

	PAH Emissions (µg PAH/g Particulate Extract)						
Compound	**Diesel 1**	**Diesel 2**	**Diesel 3**	**Diesel 4**	**Diesel 5**	**Gas 1**	**Gas 2**
Fluoranthene	1400	7300	3400	3700	540	970	2000
Pyrene	1700	8000	3700	3500	450	1800	3700
Chrysene/Triphenylene	-	1500	660	870	-	220	270
Benzo[ghi]perylene	130	420	220	230	-	51	770
Benz[a]anthracene	530	1100	460	500	48	62	91
Benzo[a]pyrene	39	560	270	210	8.0	32	260
Dibenz[a,h]anthracene	-	96	50	50	-	2.3	-
Coronene	-	520	300	310	-	340	390

C.3.1.6 Composition of Individual Mixtures: Summary

Section C.3.1 has addressed objectives 1 through 3 listed in the introduction (see section C.1). The objectives are briefly summarized as follows.

Objective 1: Determine the differences in total PAH content which exist among mixtures from the same type of source and among mixtures from different sources.

Objective 2: Determine what effect changes in operating conditions has on the levels of PAH emitted from a source.

Objective 3: Determine what effect changes in fuel have on the levels of PAH emitted from a source.

The conclusions arising from each of these objectives are discussed in the following paragraphs.

Objective 1:

The data presented in sections C.3.1.1 through C.3.1.5 demonstrate that differences in the levels of PAH among various source mixtures can be as large as six orders of magnitude (see table C.3.1.5.2). Among mixtures from the same type of source, the differences are smaller, usually less than two and frequently less than 10 orders of magnitude (see sections C.3.1.1 through C.3.1.3). It should be noted that these comparisons are limited by differences in the reporting format which make comparisons among all mixtures difficult. In addition, differences in the sampling technique which may alter PAH level detection have not been addressed in detail. Therefore, conclusions drawn must be viewed with caution.

Objective 2:

The levels of PAH emitted from a given source, such as coal derived sources, wood burning stoves, forest fires and diesel engines, can be altered by changes in the operating conditions (see sections C.3.1.1.2 and C.3.1.1.4). Such changes include changes in the production processes (high and low temperature formation) in the case of coal tar, (see section C.3.1.1.2), changes in firing systems and pollution controls for coal-fired power plants (see section C.3.1.1.4), and altering the burn conditions for wood-burning stoves and forest fires (see sections C.3.1.2.1 and C.3.1.2.2). In the situation with diesel engine exhaust, emissions of nitro-substituted PAH vary according to variation in the engine speed or load. Furthermore, a number of diesel engines operating under similar conditions were reported to emit widely varying levels of B[a]P (see section C.3.1.3.1).

For other source mixtures, changes in operating conditions have only a small effect on PAH emissions. Examples are coal stoves (different stove type, see C.3.1.1.5.2), gasoline engine emissions (C.3.1.3.2), oil and coal-fired power plants (see section C.3.1.1.4 and C.3.1.3.3).

In summary, the data suggest that changes in operating/burn conditions can alter the levels of PAH emitted from a given source. However, the magnitude of the change is dependent on individual sources.

Objective 3:

The effect of changes in fuel on the levels of PAH emitted from a source has been examined for coal and wood-burning stoves (see sections C.3.1.1.5 and C.3.1.2.2). For coal stoves, changes in the fuel type can alter the levels of PAH emitted by up to five orders of magnitude (see table C.3.1.1.5.1). In the case of wood-burning stoves, softwood tends to produce higher levels of PAH than hardwood (see tables C.3.1.2.2.1 and C.3.1.2.2.2). The differences are, however, usually less than ten-fold. For the other mixtures considered in Section C.3.1, the available information is insufficient to properly evaluate the effect of fuel changes on PAH emission.

Summary

The data reviewed in sections C.3.1.1 through C.3.1.5 show that differences in the levels of PAH exist among the various mixtures considered. However, proper quantitative comparisons cannot be made due to differences in sampling techniques and reporting formats. In addition, differences in operating conditions and fuel tend to alter the levels of PAH emitted. The present data are not sufficient to allow full evaluation of the effect of each of the factors. Nevertheless, the data suggest that the extent of changes depends on the individual source mixture and each mixture has to be evaluated individually.

C.3.2 Complex Mixtures PAH Levels Relative to B[a]P (PAH Profiles)

As discussed in section C.3.1, differences in reporting formats makes comparison of PAH content of source mixtures difficult. Furthermore, differences in operating conditions and sampling protocols appear to affect the absolute levels of PAH detected. For example, samplers placed at the stack outlet could be expected to detect higher levels of PAH than samplers removed from the immediate source. Therefore, in order to facilitate comparison of the various mixtures, it is necessary to standardize the expression of PAH content. The use of PAH profiles, expressed as PAH levels relative to a given marker, overcomes the problem of differing reporting formats and absolute levels, and allows direct comparison of PAH containing mixtures. Benzo[a]pyrene is chosen as the marker PAH because it is one of the most commonly reported PAH, and because it is one of the more biologically active PAH (see main document). Section C.3.2 aims at determining the differences in PAH profiles among mixtures from the same source type and among mixtures from different sources and, if possible, at identifying what effects changes in fuel or operating conditions have on these profiles (objectives 4 through 7 described in section C.1). The PAH profiles relative to B[a]P for all but one of the mixtures considered in section C.3.1 are presented in sections C.3.2.1 through C.3.2.4. The PAH emission data from baffled and non-baffled wood-burning stoves (section C.3.1.2.2) do not include levels for B[a]P and therefore could not be included in the analysis. Section C.3.2.6 provides a summary of the data and discusses objectives 4 through 7 individually.

A large number of PAH are reported in the various mixtures discussed in section C.3.1, but not all compounds are reported for all mixtures. Therefore, to compare the PAH profiles of the various mixtures, it is necessary to identify a set of PAH which is common to all mixtures. Table C.3.2.1 lists a number of PAH which are commonly reported in source mixtures. Fluoranthene, benzofluoranthenes and pyrene are the only PAH which are reported in all 14 of the source mixtures studied. The other compounds in table C.3.2.1 are reported in at least 6 of the 14 mixtures studied. Therefore, PAH profiles are provided for as many PAH as are found in each of the source mixtures discussed in section C.3.1. Chrysene and triphenylene are reported together because these compounds are difficult to separate and

to quantify individually, and they are usually reported together (Bjørseth *et al.* 1978). Therefore, our analysis includes studies which reported chrysene only, as it is likely that the samples also contained triphenylene. Where chrysene and triphenylene were reported individually, the values were summed. Also, there is little consistency in the reporting of the levels of individual benzofluoranthenes. Some studies provide levels for individual benzofluoranthenes while other studies present levels of individual and combined compounds. In order to reduce the confusion, the values were combined and the total reported as benzofluoranthenes. The remaining PAH, cited in section C.3.1, but excluded from table C.3.21, have been reported in only one or two source mixtures. Therefore they were not included in the analysis.

Table C.3.2.1 PAH commonly reported in PAH containing source mixtures.

Compound	No. Of Mixtures[1]
Anthracene	7
Phenanthrene	7
Fluoranthene	14
Pyrene	14
Benz[a]anthracene	12
Perylene	12
Benzo[e]pyrene	11
Benzo[a]pyrene	12
Benzo[ghi]perylene	12
Dibenz[a,h]anthracene	10
Coronene	10
Indeno[1,2,3-cd]pyrene	9
Anthanthrene	5
Chrysene/Triphenylene	12
Benzofluoranthenes	14

1) A total of 14 mixtures were considered.

For each source mixture, the PAH profile data are assembled in two tables. The first table contains the levels of individual PAH extracted from the corresponding tables in section C.3.1, and the levels relative to the B[a]P content of the sample. Where more than two data sets are available for a given source mixture, the geometric means of the relative levels were calculated for each PAH. In addition to the means, the upper and lower 95% confidence limits and the confidence range (discussed below) were calculated for each PAH. This information is contained in the second table.

Table C.3.2.2 Summary of confidence ranges from source mixtures data (sections C.3.2.1 - C.3.2.4).

(all values are rounded to two significant figures)

Compound	C.O.	C.T.	CFPP	C.S.	O&FF	W.S.	Dies	Gas	RA	PA
Anthracene	3.2	440	830	-	-	7.6	-	1.8	-	-
Phenanthrene	1.3	32	27	-	-	2.7	-	2.3	-	-
Fluoranthene	2.7	4.5	3.2	43	4.9	1.4	6.1	3.5	5.7	5.2
Pyrene	2.6	280	37	18	5.6	1.5	5.3	4.0	19	2.7
Benz[a]anthracene	19	-	2.8	32	160	2.0	9.0	-	130	8.5
Perylene	2.3	27	-	23	8.2	31	-	4.8	4.6	5.9
Benzo[e]pyrene	1.3	5.5	3.3	35	1.6	1.5	2.2	1.8	-	-
Benzo[ghi]perylene	8.8	-	-	12	32	2.7	8.9	1.7	5.1	2.8
Dibenz[a,h]anthracene	-	-	-	310	3.2	-	2.4	-	-	-
Coronene	7.8	-	-	7.1	730	-	3.2	1.9	-	-
Indeno[1,2,3-cd]pyrene	-	-	-	-	7.4	1.9	7.0	1.8	-	-
Anthanthrene	8.7	-	-	-	-	-	-	2.1	-	-
Chrysene/Triphenylene	2.1	12	430	42	25	4.3	7.3	2.4	23	6.1
Benzofluoranthenes	5.7	-	2.0	28	7.1	5.0	3.4	1.7	7.7	120

The confidence range (CR) is used as a measure of the variation in the relative levels of individual PAH among samples from a single source mixture. The CR is determined by dividing the Upper 95% confidence limit by the Lower 95% confidence limit. All CR values which could be calculated from the data in sections C.3.2.1 through C.3.2.4 are summarized in table C.3.2.2. These data have been grouped according to the magnitude of CR (see table C.3.2.3). A total of 95 values are available. The data in table C.3.2.3 are arranged to show the number of samples which exceed the indicated CR values and presented as a percentage of the total number of samples. A plot of the data shows that the CR is less than 50 for 90% of the samples and 6.0 or less for 50% of the samples listed in table C.3.2.2 (see Figure C.3.2.1).

The result indicates that the variation in the levels of PAH, relative to B[a]P, among samples from the same source mixture is small. In addition, the size of this variation is unlikely to be large enough to have a significant effect on the estimation of risk or on the uncertainty associated with risk estimation. Therefore, a CR value of less than 50 was selected to indicate that variation in PAH levels is unlikely to alter the estimation of risk and to represent PAH levels which can be considered stable. Additionally, CR values of 50 or less suggest that the relative levels of PAH are sufficiently stable that the level of B[a]P could be used to predict, with a reasonable degree of accuracy, the levels of other PAH in a given source mixture.

Table C.3.2.3 Distribution of confidence ranges for all source mixtures.

CR	Number	% Total
> 1.0	95	100
> 2.0	82	86
> 3.0	66	69
> 4.0	59	62
> 5.0	53	56
> 6.0	44	46
> 7.0	42	44
> 8.0	34	36
> 9.0	29	31
> 10	28	29
> 20	23	24
> 30	17	19
> 40	11	12
> 50	9	9.5
> 100	9	9.5
> 200	6	6.3
> 500	2	2.1
> 1000	0	0.0

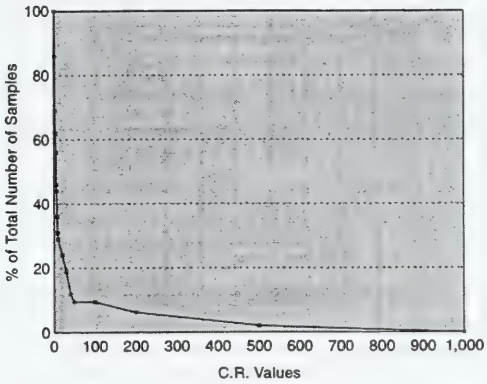

Figure C.3.2.1 Distribution of confidence ranges for all source mixtures.

Table C.3.2.4 Distribution of confidence ranges for individual PAH.

CR Range	Anth	Phen	Flan	Pyr	B[a]A	Pery	B[e]P	BghiP	DBahA	Coro	IP	Antha	C/T	Bflans
1 ≤ CR < 2	1	1	1	1			4	1		1	2			1
2 ≤ CR < 3		2	1	2	2	1	1	2	1			1	2	1
3 ≤ CR < 4	1		2				1		1	1				1
4 ≤ CR < 5			2	1		2							1	
5 ≤ CR < 6			2	2		1	1	1						2
6 ≤ CR < 7			1										1	
7 ≤ CR < 8	1									2	2		1	2
8 ≤ CR < 9					1	1		2				1		
9 ≤ CR < 10					1									
10 ≤ CR < 20				2	1			1					1	
20 ≤ CR < 30		1				2							2	1
30 ≤ CR < 40		1		1	1	1	1	1						
40 ≤ CR < 50			1										1	
50 ≤ CR < 100														
100 ≤ CR < 200					2									1
200 ≤ CR < 500	1			1					1				1	
500 ≤ CR < 1000	1									1				

Tables C.3.2.2 and C.3.2.3 provide a general summary of the range of CR values which are found across the entire group of PAH examined. They do not, however, indicate if there are any specific PAH which have a higher proportion of larger CR values. The latter information is addressed in table C.3.2.4 where the number of CR values which fall within each indicated range is shown for each PAH. As illustrated in the table, variation in CR values are similar for all PAH listed, and no one PAH appears to have significantly higher CR values than other compounds. Whether these variations are distributed evenly across the source mixtures, and whether some mixtures show a greater degree of variation in the relative levels of PAH are examined in sections C.3.2.1 through C.3.2.4. Finally, section C.3.2.5 compares the CR ranges and variation in PAH profiles for all the mixtures,

C.3.2.1 PAH Profiles in Coal Derived Source Mixtures

The levels of numerous PAH identified in the various coal derived source mixtures are presented in section C.3.1.1. The following subsections will provide the PAH levels relative to B[a]P for each of the mixtures considered in section C.3.1.1.

C.3.2.1.1 PAH Profiles in Coke Oven Emissions

Levels of the selected PAH in coke oven battery top emissions and their levels relative to B[a]P are shown in table C.3.2.1.1.1. These values were used to calculate the mean, upper and lower 95% confidence limits and the confidence range (see table C.3.1.1.1.2). The confidence ranges were found to be less than 20 for all compounds. The largest value was reported for benz[a]anthracene (CR = 19), while for many of the compounds, the CR values were less than 3.0. This finding suggests that the relative levels of these PAH vary little between the spring and the fall and that B[a]P levels could be used as adequate predictors for the levels of these compounds. It is therefore unlikely that variation in the relative levels of these compounds will be sufficient to have an effect on the estimation of risk.

Table C.3.2.1.1.1 PAH levels in coke oven emissions.

(all values are rounded to two significant figures)

	PAH Levels µg PAH/m³							
	Spring				Fall			
	Minimum		Maximum		Minimum		Maximum	
Compound	µg/m³	R.L.[1]	µg/m³	R.L.	µg/m³	R.L.	µg/m³	R.L.
Anthracene	58	2.4	330	2.4	16	1.1	160	2.3
Phenanthrene	170	7.1	960	7.1	82	5.9	490	6.9
Fluoranthene	150	6.3	430	3.2	46	3.3	280	3.9
Pyrene	110	4.6	320	2.41	35	2.5	210	3.0
Benz[a]anthracene	5.4	0.23	160	1.2	23	1.6	110	1.5
Perylene	5.8	0.25	19	0.14	3.3	0.24	16	0023
Benzo[e]pyrene	15	0.63	73	0.54	8.0	0.57	36	0.51
Benzo[ghi]perylene	8.7	0.36	23	0.17	11	0.79	45	0.63
Dibenz[a,h]anthracene	-	-	-	-	-	-	-	-
Coronene	6.7	0.28	24	0.18	1.0	0.070	19	0.27
Indeno[1,2,3-cd]pyrene	-	-	-	-	-	-	-	-
Anthanthrene	6.4	0.27	33	0.24	2.6	0.19	62	0.87
Chrysene/Triphenylene	27	1.1	190	1.4	26	1.9	130	1.8
Benzofluoranthenes	13	0.54	100	0.76	5.5	0.39	100	1.5
Benzo[a]pyrene	24		135		14		71	

1) Level relative to B[a]P.

Table C.3.2.1.1.2 Confidence range analysis for coke oven emissions.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]
Anthracene	2.0	3.5	1.1	3.2
Phenanthrene	6.7	7.8	5.8	1.3
Fluoranthene	4.0	6.6	2.4	2.7
Pyrene	3.0	4.8	1.9	2.6
Benz[a]anthracene	0.91	4.0	0.21	19
Perylene	0.21	0.32	0.14	2.3
Benzo[e]pyrene	0.56	0.65	0.49	1.3
Benzo[ghi]perylene	0.42	1.2	0.14	8.8
Dibenz[a,h]anthracene	-	-	-	-
Coronene	0.18	0.49	0.063	7.8
Indeno[1,2,3-cd]pyrene	-	-	-	-
Anthanthrene	0.32	0.95	0.11	8.7
Chrysene/Triphenylene	1.5	2.2	1.0	2.1
Benzofluoranthenes	0.68	1.6	0.29	5.7

1) Confidence range.

C.3.2.1.2 PAH Profiles in Coal-Tars

The levels of PAH in both LTCT and HTCT and the PAH profiles are shown in table C.3.2.1.2.1. As indicated in section C.3.1.1.2, the LTCT and HTCT mixtures differ in their four to seven ring PAH content. As a consequence, LTCT and HTCT should be considered as separate mixtures. Since only one set of data is available for LTCT, geometric means, therefore, could not be calculated. This data set is, however, included in the comparison of all mixtures (see section C.3.2.5). The means, upper and lower 95% confidence limits and the confidence ranges, calculated for the HTCT data are presented in table C.3.2.1.2.2.

The confidence range, CR, values for five of the seven PAH are lower than 40, suggesting that the levels of these compounds, relative to B[a]P, do not vary sufficiently to affect the estimation of risk. In addition, the B[a]P levels would be good predictors for the levels of these compounds in the mixture. For the remaining two PAH, anthracene and pyrene, the CR values exceed 50. The large CR values may, in part, be explained by the small number of samples used

to calculate the mean and CR. The confidence range is a function of the *standard error* of the mean. Thus the size of the confidence range (CR) is a function of the number of samples used. Because only three points were used to calculate the mean and upper and lower 95% confidence limits, any outlying points could be expected to have a larger effect than if a larger sample size were used.

Table C.3.2.1.2.1 PAH levels relative to B[a]P in coal tars.

(all values are rounded to two significant figures)

	PAH Levels mg PAH/g Tar							
	LTCT		HTCT1		HTCT2		HTCT3	
Compound	mg/g tar	R.L.[1]	mg/g tar	R.L.	mg/g tar	R.L.	mg/g tar	R.L.
Anthracene	0.60	7.5	15	2.7	55	14	12	1.2
Phenanthrene	16	200	50	9.1	87	13	53	5.5
Fluoranthene	0.18	2.3	33	6.0	30	8.0	40	4.1
Pyrene	0.11	1.4	21	3.8	99	25	31	3.2
Benz[a]anthracene	0.14	1.8	6.5	1.2	-	-	8.0	0.82
Perylene	0.11	1.4	2.5	.045	5.5	1.4	4.2	0.43
Benzo[e]pyrene	-	-	5.0	0.91	5.0	1.3	6.1	0.63
Benzo[ghi]perylene	-	-	5.5	1.0	-	-	7.0	0.72
Dibenz[a,h]anthracene	-	-	1.0	0.18	-	-	-	-
Coronene	-	-	-	-	-	-	2.0	0.21
Indeno[1,2,3-cd]pyrene	-	-	5.0	0.91	-	-	8.0	.082
Anthanthrene	-	-	-	-	-	-	-	-
Chrysene/Triphenylene	0.09	1.1	12	2.2	24	6.0	11	1.1
Benzofluoranthenes	0.14	1.8	9.0	1.8	-	-	11	1.1
Benzo[a]pyrene	0.080		5.5		4.0		9.7	

1) Level relative to B[a]P.

Table C.3.2.1.2.2 Confidence range analysis for high temperature coal tars.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	3.6	76	0.17	440	*
Phenanthrene	10	58	1.8	32	
Fluoranthene	5.7	12	2.7	4.5	
Pyrene	6.7	110	0.40	280	*
Benz[a]anthracene	0.99	-	-	-	
Perylene	0.64	3.3	0.12	27	
Benzo[e]pyrene	0.89	2.1	0.38	5.5	
Benzo[ghi]perylene	0.85	-	-	-	
Dibenz[a,h]anthracene	0.18	-	-	-	
Coronene	0.21	-	-	-	
Indeno[1,2,3-cd]pyrene	0.86	-	-	-	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	2.0	7.1	0.58	12	
Benzofluoranthenes	1.4	-	-	-	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

In general, the CR values for most of the PAH tested are less than 40, suggesting that the PAH profiles remain relatively constant among HTCT samples. The CR values for anthracene and pyrene (440 and 280, respectively) could reasonably be expected to decrease (possibly below 50) if the number of samples were increased. Therefore, one can conclude that the PAH profiles for HTCT are sufficiently stable that variation in the profiles are unlikely to have an effect on the estimation of risk.

C.3.2.1.3 PAH Profiles from Iron and Steel Foundries

Emissions from iron and steel foundries have been considered in section C.3.1.1.3. Table C.3.2.1.3.1 reports the PAH levels and their levels relative to B[a]P. The available data are insufficient to evaluate the variation among samples. Therefore, the mean, upper and lower 95% confidence limits and CR could not be calculated.

Table C.3.2.1.3.1 PAH levels relative to B[a]P from iron and steel foundries.

(all values are rounded to two significant figures)

Compound	µg PAH/m^3	R.L.[1]
Anthracene	2.3	4.9
Phenanthrene	4.5	9.6
Fluoranthene	1.6	3.4
Pyrene	1.7	3.6
Benz[a]anthracene	0.67	1.4
Perylene	0.21	0.45
Benzo[e]pyrene	0.48	0.10
Benzo[ghi]perylene	0.72	1.5
Dibenz[a,h]anthracene	0.24	0.43
Coronene	0.21	0.45
Indeno[1,2,3-cd]pyrene	0.81	1.7
Anthanthrene	0.64	1.4
Chrysene/Triphenylene	0.82	1.7
Benzofluoranthenes	0.87	1.9
Benzo[a]pyrene	0.47	

1) Level relative to B[a]P.

C.3.2.1.4 PAH Profiles from Coal-Fired Power Plants

The levels of PAH in flue-gas emissions from various coal-fired power plants have been reviewed in section C.3.1.1.4. In many of the samples, the levels of B[a]P and other PAH were below the minimum detection limits (MDL). Therefore, these data could not be used to determine PAH profiles. Only four samples had measurable PAH levels. The PAH levels and the relative levels for these four samples are shown in table C.3.2.1.4.1. Table C.3.2.1.4.2 provides the means, upper and lower 95% confidence limits and confidence range calculated from these relative levels. The CR values for anthracene and chrysene/triphenylene both exceed 50 (830 and 430,

respectively), suggesting that the variation in the levels of these PAH is large. The remaining six PAH, for which CR values could be calculated, all have CR values below 50 and many have values below 5. Thus, the relative levels of these compounds are unlikely to vary sufficiently to have an effect on the estimation of risk. In addition, the levels of B[a]P may serve as a good predictor for the levels of these compounds in the source mixture. The small sample size (4 data points) may account for the variation in the levels of anthracene and chrysene/triphenylene. It is reasonable to expect that a larger number of samples would result in lower CR values for both of these compounds (see section C.3.2.1.2). In general, the profiles could be considered to be sufficiently stable that it is unlikely that risk estimates will be altered by changes in the levels of individual PAH.

Table C.3.2.1.4.1 PAH levels relative to B[a]P from coal-fired power plants.

(all values are rounded to two significant figures)

	Plant A		Plant B		Plant G		Plant H	
Compound	**µg PAH/m³**	**R.L.[1]**	**µg PAH/m³**	**R.L.**	**µg PAH/m³**	**R.L.**	**µg PAH/m³**	**R.L.**
Anthracene	0.040	0.57	0.050	2.5	-	-	110	8.7
Phenanthrene	0.37	5.3	0.85	43	15	19	660	53
Fluoranthene	0.80	11	0.38	19	22	28	230	18
Pyrene	0.31	4.4	0.18	9.0	0.911	1.2	210	16
Benz[a]anthracene	0.080	1.1	0.040	2.0	1.4	1.8	32	2.6
Perylene	-	-	-	-	-	-	2.1	0.17
Benzo[e]pyrene	0.080	1.1	0.010	0.50	0.79	1.0	13	1.0
Benzo[ghi]perylene	-	-	-	-	-	-	3.9	0.31
Dibenz[a,h]anthracene	-	-	-	-	-	-	1.0	0.080
Coronene	-	-	-	-	-	-	-	-
Indeno[1,2,3-cd]pyrene	-	-	-	-	-	-	5.0	0.40
Anthanthrene	-	-	-	-	-	-	-	-
Chrysene/Triphenylene	0.37	5.3	0.19	9.5	0.10	0.13	18	1.5
Benzofluoranthenes	0.23	3.2	0.05	2.5	2.3	2.5	26	2.1
Benzo[a]pyrene	0.070		0.02		0.79		13	

1) Level relative to B[a]P.

Table C.3.2.1.4.2 Confidence range analysis for coal-fired power plants.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	2.3	66	0.080	830	*
Phenanthrene	22	110	4.2	27	
Fluoranthene	18	32	10	3.2	
Pyrene	5.2	32	0.89	37	
Benz[a]anthracene	1.8	3.0	0.85	2.8	
Perylene	0.017	-	-	-	
Benzo[e]pyrene	0.87	1.6	0.48	3.3	
Benzo[ghi]perylene	-	-	-	-	
Dibenz[a,h]anthracene	0.080	-	-	-	
Coronene	-	-	-	-	
Indeno[1,2,3-cd]pyrene	0.40	-	-	-	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	1.7	36	0.084	430	*
Benzofluoranthenes	2.6	3.7	1.9	2.0	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.1.5 PAH Profiles from Residential Coal Stoves

The emissions from coal-burning residential stoves have been reported in section C.3.1.1.5. The relative levels of these PAH are summarized in table C.3.2.1.5.1. These profile data were used to calculate the mean, upper and lower 95% confidence limits and the CR for each PAH (see table C.3.2.1.5.2). Of the ten compounds examined, dibenz[a,h]anthracene is the only PAH for which the CR exceeds 50. The CR values for the remaining nine PAH are all less than 50, but only one CR value is less than 10. This observation suggests that the PAH profiles are less stable in emissions from coal stoves than in other source mixtures where the CR values for many PAH are less than 10. The larger variation in coal stove emissions is likely due to differences in the types of coal used. These differences are discussed in detail in section C.3.1.1.5. Although the PAH profiles of the residential coal stove emissions appear to be less stable than other source mixtures, the variation in relative PAH levels are unlikely to be large enough to have a significant effect on the estimation of risk. The data also indicate that the levels of B[a]P could be used as an indicator for the levels of other PAH, with the exception of dibenz[a,h]anthracene, in coal stove emissions.

C.3.2.1.6 PAH Profiles in Coal-Derived Source Mixtures: Summary

The PAH profiles for a number of coal-derived source mixtures have been examined in sections C.3.2.1.1 through C.3.2.1.5. Although PAH profiles could be calculated for each mixture, confidence range analysis could not be conducted for the iron and steel foundry data as only a single data set was available (see section C.3.2.1.3). The CR analysis for the majority of the PAH considered in the various mixtures shows that the PAH profiles remain constant within the limit established (CR < 50). For a few PAH in some of the mixtures, CR values exceed the limit of 50, suggesting that the levels of these compounds may not be stable. However, there is no clear pattern for the compounds which exceed the CR 50 value. For example, the CR value for anthracene exceeds 50 in coal-tars and coal-fires power plants (440 and 830, respectively), but is below 50 in coke oven emissions (3.2). The CR value for pyrene exceeds 50 in coal-tar (280) but are below 50 in the other mixtures. The studies used provide insufficient information to determine why the CR values for these compounds exceed 50 in some mixtures but not all. It is not possible to determine what contribution differences in fuel or operating conditions make to these values. It should be noted that for the majority of the PAH, CR values in all mixtures are below 50 and that there is no consistent pattern for compounds with CR values that exceed 50. This observation suggests that, on a weight of evidence basis, PAH profiles from coal-derived sources could be considered to remain constant.

Table C.3.2.1.5.1 PAH levels relative to B[a]P in emissions from coal-burning stoves.

(all values are rounded to two significant figures)

	Anthracite Briq.		Lignite Briq.		Anthracite		Broken Coke		*Anzit*		*Extrazit*		Slow Combustion Stove		Permanent Combustion Stove	
Compound	µg/m³	R.L.[1]	µg/m³	R.L.	µg/m³	R.L.	µg/m³	R.L.	µg/m³	R.L.	µg/m³	R.L.	µg/m³	R.L.	µg/m³	R.L.
Anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenanthrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fluoranthene	380	5.8	70	14	0.087	7.3	-	-	3.9	1800	0.028	56	3.3	7.2	15	13
Pyrene	87	1.3	15	3.0	0.025	2.1	0.0017	0.17	0.13	59	0.0044	8.8	3.0	6.5	12	10
Benz[a]anthracene	160	2.5	21	4.2	0.069	5.8	0.0087	0.87	1.8	820	0.0045	9.0	1.1	2.4	3.5	2.9
Perylene	140	2.2	13	2.6	0.023	1.9	0.017	1.7	0.14	64	-	-	0.20	0.43	0.44	0.37
Benzo[e]pyrene	200	3.1	34	6.8	0.19	16	1.2	120	0.68	310	0.032	64	0.48	1.0	1.6	1.3
Benzo[ghi]perylene	96	1.5	20	4.0	0.027	2.3	0.12	12	0.073	33	0.0051	10	0.35	0.76	0.50	0.42
Dibenz[a,h]anthracene	23	0.35	10	2.0	0.013	1.1	-	-	0.054	25	-	-	-	-	-	-
Coronene	41	0.63	6.3	1.3	0.0076	0.63	0.0058	0.58	0.011	5.0	0.0016	3.2	-	-	-	-
Indeno[1,2,3-cd]pyrene	-	-	-	-	-	-	-	-	-	-	-	-	0.21	0.46	0.55	0.46
Anthanthrene	-	-	-	-	-	-	-	-	-	-	-	-	0.040	0.087	0.080	0.067
Chrysene/Triphenylene	160	2.5	16	3.2	0.11	9.2	0.019	19	3.5	1600	0.018	36	1.6	3.5	5.4	4.5
Benzofluoranthenes	280	4.2	18	3.6	0.19	15	0.078	7.8	1.9	870	0.017	33	0.95	2.1	3.0	2.5
Benzo[a]pyrene	65		5.0		0.012		0.010		0.0022		0.00050		0.46		1.2	

1) Level relative to B[a]P.

Table C.3.2.1.5.2 Confidence range analysis for coal-burning stoves.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	-	-	-	-	
Phenanthrene	-	-	-	-	
Fluoranthene	25	160	3.7	43	
Pyrene	3.8	16	0.92	18	
Benz[a]anthracene	6.4	36	1.1	32	
Perylene	2.1	10	0.44	23	
Benzo[e]pyrene	13	80	2.3	35	
Benzo[ghi]perylene	3.4	12	0.98	12	
Dibenz[a,h]anthracene	2.1	36	0.12	310	*
Coronene	1.3	3.4	0.49	7.1	
Indeno[1,2,3-cd]pyrene	4.6	-	-	-	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	10	67	1.6	42	
Benzofluoranthenes	11	60	2.2	28	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.2 PAH Profiles in Wood Derived Source Mixtures

The levels of various PAH, identified in some wood-derived source mixtures have been presented in sections C.3.1.2.1 and C.3.1.2.2. The following subsections provide the levels of these PAH and their concentrations relative to B[a]P in wood-derived source mixtures. The mean, upper and lower 95% confidence limits and confidence ranges are also provided for each PAH.

Table C.3.2.2.1.1 PAH levels relative to B[a]P in open and forest fires.

(all values are rounded to two significant figures)

	Open Fires (µg PAH/g Particulate)						Simulated Backing Fires (µg PAH/g Particulate)						Simulated Heading Fire (µg PAH/g Particulate)					
	Fireplace		Open Fire		Bush Fire		0.1 lb/ft^3		0.3 lb/ft^3		0.5 lb/ft^3		0.1 lb/ft^3		0.3 lb/ft^3		0.5 lb/ft^3	
Compound	µg/g	R.L.[1]	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.
Anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenanthrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fluoranthene	88	3.3	200	1.1	70	099	1300	4.2	480	4.0	270	2.8	73	1.9	26	24	18	11
Pyrene	100	3.8	130	0.68	68	0.96	1900	6.2	690	5.8	430	4.5	110	2.9	26	24	19	12
Benz[a]anthracene	39	1.4	38	0.20	60	0.85	-	-	-	-	-	-	-	-	-	-	-	-
Perylene	-	-	-	-	-	-	190	0.61	44	0.37	54	0.57	33	0.87	6.5	5.9	0.78	0.49
Benzo[e]pyrene	-	-	-	-	-	-	530	1.7	260	2.2	270	2.8	61	1.6	2.1	1.9	2.6	1.6
Benzo[ghi]perylene	5.8	0.21	8.2	0.043	10	0.14	560	1.8	220	1.8	170	1.8	-	-	-	-	-	-
Dibenz[a,h]anthracene	1.2	0.044	12	0.063	4.3	0.061	-	-	-	-	-	-	-	-	-	-	-	-
Coronene	0.70	0.026	26	0.14	1.0	0.014	-	-	-	-	-	-	-	-	-	-	-	-
Indeno[1,2,3-cd]pyrene	10	0.37	20	0.11	20	0.28	390	1.3	150	1.3	68	0.72	-	-	-	-	-	-
Anthanthrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chrysene/Triphenylene	63	2.3	130	0.68	64	0.90	-	-	-	-	-	-	-	-	-	-	-	-
Benzofluoranthenes	51	1.9	27	0.14	16	0.23	1200	3.9	270	2.3	330	3.5	16	0.42	3.5	3.2	4.1	2.6
Benzo[a]pyrene	27		190		71		310		120		95		38		1.1		1.6	

1) Level relative to B[a]P.

C.3.2.2.1 PAH Profiles in Forest and Open Fires

The PAH levels in emissions from forest fires and various types of open fires have been discussed in section C.3.1.2.1. The levels of the PAH relative to B[a]P are presented in table C.3.2.2.1.1. These data were used to calculate the mean, upper and lower 95% confidence limits and the confidence range for each PAH (see table C.3.2.2.1.2). The CR values for nine of the eleven PAH are below 35, suggesting that the PAH levels of these compounds, relative to B[a]P, do not vary sufficiently to alter the estimation of risk. The finding also indicates that the level of B[a]P could be used to estimate the levels of these PAH in the mixtures. The CR values for benz[a]anthracene and coronene (160 and 730, respectively) both exceed 50. However, only three data points were used to calculate the CR values in both cases. It is possible that larger data sets would show smaller confidence ranges for both compounds. Therefore, it is possible to infer that the levels of the PAH listed in table C.3.2.2.1.2, relative to B[a]P, are unlikely to change sufficiently to have an effect on the estimated risk.

Table C.3.2.2.1.2 Confidence range analysis for open wood burning.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	-	-	-	-	
Phenanthrene	-	-	-	-	
Fluoranthene	3.5	7.8	1.6	4.9	
Pyrene	4.1	9.7	1.7	5.6	
Benz[a]anthracene	0.63	7.9	0.050	160	*
Perylene	0.83	2.4	0.29	8.2	
Benzo[e]pyrene	1.9	2.4	1.5	1.6	
Benzo[ghi]perylene	0.44	2.5	0.078	32	
Dibenz[a,h]anthracene	0.052	0.094	0.029	3.2	
Coronene	0.035	0.94	0.0013	730	*
Indeno[1,2,3-cd]pyrene	0.48	1.3	0.18	7.4	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	1.1	5.6	0.23	25	
Benzofluoranthenes	1.2	3.3	0.46	7.1	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.2.2 PAH Profiles from Residential Wood Burning Stoves

The levels of PAH emitted from residential wood-burning stoves were discussed in section C.3.1.2.2. Of the two data sets presented, one data set did not provide levels of B[a]P and could not be used in subsequent calculations. Data from the remaining study was used to calculate the levels of PAH relative to B[a]P (see table C.3.2.2.2.1). The mean, upper and lower 95% confidence limits and the CR were also calculated for each compound listed (see table C.3.2.2.2.2). The CR values for all the compounds listed are below 50 and for ten of the eleven compounds, the CR values are less than 10. The observation indicates that the levels of the PAH, relative to B[a]P, could be considered to be stable. Any variation in the levels of these compounds is not likely to be of sufficient magnitude to affect the estimation of risk. In addition, this stability means that the level of B[a]P can be used to predict, with a reasonable degree of accuracy, the levels of these compounds in a similar mixture .

Table C.3.2.2.2.1 PAH levels relative to B[a]P from wood-burning stoves.

(all values are rounded to two significant figures)

	Birch Normal		Birch Air Starved		Spruce Normal		Spruce Air Starved	
Compound	µg PAH/m³	R.L.[1]	µg PAH/m³	R.L.	µg PAH/m³	R.L.	µg PAH/m³	R.L.
Anthracene	87	1.5	95	6.8	14	2.6	170	3.0
Phenanthrene	620	10	290	21	96	18	780	14
Fluoranthene	300	5.0	76	5.4	34	6.4	300	5.3
Pyrene	290	4.8	85	6.1	27	5.1	350	6.1
Benz[a]anthracene	51	0.85	19	1.4	6.9	1.3	72	1.3
Perylene	29	0.48	1.4	0.10	2.6	0.49	3.5	0.61
Benzo[e]pyrene	37	0.62	9.5	0.68	4.0	0.75	32	0.56
Benzo[ghi]perylene	58	0.97	11	0.79	3.9	0.73	26	0.46
Dibenz[a,h]anthracene	-	-	-	-	-	-	-	-
Coronene	-	-	-	-	-	-	-	-
Indeno[1,2,3-cd]pyrene	44	0.73	9.7	0.69	3.8	0.72	27	0.47
Anthanthrene	-	-	-	-	-	-	-	-
Chrysene/Triphenylene	65	1.1	27	1.9	-	-	77	1.4
Benzofluoranthenes	110	1.8	10	0.71	12	2.3	94	1.6
Benzo[a]pyrene	60		14		5.3		57	

1) Level relative to B[a]P.

Table C.3.2.2.2.2 Confidence range analysis for wood-burning stoves.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	3.0	8.2	1.1	7.6	
Phenanthrene	15	25	9.3	2.7	
Fluoranthene	5.5	6.5	4.6	1.4	
Pyrene	5.5	6.7	4.5	1.5	
Benz[a]anthracene	1.2	1.7	0.83	2.0	
Perylene	0.19	1.1	0.035	31	
Benzo[e]pyrene	0.65	0.79	0.53	1.5	
Benzo[ghi]perylene	0.71	1.2	0.43	2.7	
Dibenz[a,h]anthracene	-	-	-	-	
Coronene	-	-	-	-	
Indeno[1,2,3-cd]pyrene	0.64	0.90	0.46	1.9	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	1.4	2.9	0.68	4.3	
Benzofluoranthenes	1.5	3.3	0.66	5.0	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.2.3 PAH Profiles in Wood-Derived Source Mixtures: Summary

The PAH profiles for a number of wood-derived source mixtures have been examined in sections C.3.2.2.1 and C.3.2.2.2. The confidence range data indicate that for the majority of the PAH, the profiles remain constant within the established limit (CR < 50). Changes in operating conditions or fuel do not appear to have a significant effect on the PAH profile. Therefore, on a weight of evidence basis, the PAH profiles from wood-derives source mixtures could be expected to remain constant.

C.3.2.3 PAH Profiles in Petroleum Derived Source Mixtures

The levels of various petroleum derived source mixtures have been presented in section C.3.1.3. The following subsections provide the PAH profile data for each of the source mixtures considered. The stability of the PAH profiles for each source mixture is examined to determine the variation in PAH profiles and whether the variation is of sufficient magnitude to effect the estimation of risk for any of the mixtures.

C.3.2.3.1 PAH Profiles in Diesel Exhaust Emissions

Table C.3.2.3.1.1 PAH levels relative to B[a]P in diesel exhaust emissions.

(all values are rounded to two significant figures)

	V#1 (µg/g extract)		VW#1 (µg/g extract)		VW#2 (µg/g extract)		VW#3 (µg/g extract)		Olds (µg/g extract)	
Compound	µg/g	R.L.[1]	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.
Anthracene	-	-	-	-	-	-	-	-	-	-
Phenanthrene	-	-	-	-	-	-	-	-	-	-
Fluoranthene	1400	36	7300	13	3400	13	3700	18	540	68
Pyrene	1700	44	8000	14	3700	14	3500	17	450	56
Benz[a]anthracene	530	14	1100	1.8	460	1.7	500	2.4	48	6.0
Perylene	-	-	-	-	-	-	-	-	-	-
Benzo[e]pyrene	-	-	950	1.7	510	1.9	490	2.3	-	-
Benzo[ghi]perylene	130	3.3	420	0.75	220	0.81	230	1.1	-	-
Dibenz[a,h]anthracene	-	-	96	017	50	0.19	50	0.24	-	-
Coronene	-	-	520	0.93	300	1.1	310	1.5	-	-
Indeno[1,2,3-cd]pyrene	-	-	93	0.17	30	0.11	50	0.24	-	-
Anthanthrene	-	-	-	-	-	-	-	-	-	-
Chrysene/Triphenylene	-	-	1500	2.7	660	2.4	870	4.1	-	
Benzofluoranthenes	-	-	2800	5.0	1100	4.0	1400	6.7	-	-
Benzo[a]pyrene	39		560		270		210		8.0	

1) Level relative to B[a]P.

Table C.3.2.3.1.2 Confidence range analysis for diesel exhaust emissions.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	-	-	-	-	
Phenanthrene	-	-	-	-	
Fluoranthene	23	58	9.5	6.1	
Pyrene	24	55	10	5.3	
Benz[a]anthracene	3.6	11	1.2	9.0	
Perylene	-	-	-	-	
Benzo[e]pyrene	2.0	2.9	1.3	2.2	
Benzo[ghi]perylene	1.2	3.7	0.41	8.9	
Dibenz[a,h]anthracene	0.20	0.31	0.13	2.4	
Coronene	1.2	2.1	0.64	3.2	
Indeno[1,2,3-cd]pyrene	0.16	0.44	0.063	7.0	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	4.1	11	1.5	7.3	
Benzofluoranthenes	5.1	9.5	2.8	3.4	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

A number of classical and nitro-substituted PAH have been identified in diesel exhaust emissions (see section C.3.1.3.1). The studies which reported the levels of the nitro-substituted PAH did not provide information on the B[a]P levels (see section C.3.1.3.1). Therefore, these studies could not be used for PAH profile analysis. The analysis has focused its attention, instead, on the classical PAH. The levels of classical PAH reported in diesel emissions and their levels relative to B[a]P are shown in table C.3.2.3.1.1. The means, upper and lower 95% confidence limits and CR values calculated from these data are provided in table C.3.2.3.1.2.

The CR values for all ten of the PAH listed in table C.3.2.3.1.2 are less than 10, indicating that the PAH profiles found in this source mixture are very stable, and that any variation in relative levels is unlikely to have an effect on the estimation of risk. The finding also suggests that the level of B[a]P would serve as a good indicator for the levels of the ten compounds which could be expected in diesel exhaust emissions. The data in section C.3.1.3.1 show that changes in operating conditions can affect the levels of PAH contained in the emissions. However, when the levels of the same compounds are compared on a relative basis, operating conditions have no appreciable effect on the PAH profiles.

C.3.2.3.2 PAH Profiles in Gasoline Engine Exhaust Emissions

PAH levels in emissions from various gasoline engines were examined in section C.3.1.3.2. The levels of PAH reported in gasoline engine emissions and their levels relative to B[a]P are provided in tables C.3.2.3.2.1a through C.3.2.3.2.1c. The relative level data were used to determine the mean , upper and lower 95% confidence limits and confidence range for each of the PAH listed (see table C.3.2.3.2.2). The CR values for each of the PAH is less than 5.0 and many are less than 2.0, indicating that the levels of these PAH, relative to B[a]P, are very stable and vary little among individual sources. Any variation which does occur among samples will probably not be large enough to alter the estimation of risk. The low CR values reported for all PAH increase the confidence in this observation. In addition, the low degree of variation means that the level of B[a]P could serve as a good predictor for the levels of the other PAH which may be present in a given mixture from gasoline engines.

Table C.3.2.3.2.1a PAH levels relative to B[a]P in gasoline engine exhaust.

(all values are rounded to two significant figures)

	Hoffmann[1]		Grimmer[2]		Alsberg *et al.*[3]		Alsberg *et al.*[3]		Hagemann *et al.*[4]		Hagemann *et al.*[4]		Hagemann *et al.*[4]		Model 1[5]		Model 2[5]	
Compound	µg/g E[6]	R.L.[7]	µg/g E[6]	R.L.	µg/mile	R.L.	µg/mile	R.L.	µg/mile	R.L.	µg/mile	R.L.	µg/mile	R.L.	µg/Eur[8]	R.L.	µg/Eur[8]	R.L.
Anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	370	9.7	220	12
Phenanthrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1200	31	690	40
Fluoranthene	970	30	2000	7.8	32	10	23	3.2	0.09	0.13	0.13	0.18	0.22	0.12	490	13	280	16
Pyrene	1800	56	37	14	45	15	50	6.9	0.18	0.26	0.20	0.28	0.37	0.19	610	16	420	24
Benz[a]anthracene	62	1.9	91	0.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perylene	-	-	55	0.21	0.48	0.15	0.80	0.11	0.22	0.31	0.33	0.46	1.6	0.84	-	-	-	-
Benzo[e]pyrene	420	13	200	0.77	4.2	1.4	10	1.4	0.88	1.3	0.72	1.0	1.5	0.79	45	1.2	19	1.1
Benzo[ghi]perylene	51	1.6	770	13	9.5	3.1	21	2.9	2.2	3.1	1.3	1.8	2.2	1.2	150	3.8	60	3.4
Dibenz[a,h]anthracene	2.3	0.072	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coronene	340	11	390	1.5	11	3.4	19	2.6	3.5	5.0	1.2	1.7	1.8	0.95	87	2.3	45	2.6
Indeno[1,2,3-cd]pyrene	82	2.6	250	0.96	2.7	0.87	5.8	0.81	1.4	2.0	1.00	1.4	2.1	1.1	37	1.0	13	0.80
Anthanthrene	44	1.4	-	-	0.32	0.10	2.1	0.29	0.22	0.31	0.15	0.21	0.15	0.079	34	0.9	8.9	0.50
Chrysene/Triphenylene	224	7.0	271	1.0	11	3.5	14	1.9	0.88	1.3	0.17	0.24	0.26	0.14	270	7.0	56	3.2
Benzofluoranthenes	135	4.2	295	1.1	8.1	2.6	13	1.8	0.53	0.76	0.55	0.76	0.90	0.47	52	1.4	18	1.0
Benzo[a]pyrene	32		260		3.1		7.2		0.70		0.72		1.9		39		18	

1) Hoffmann & Wynder (1962)
2) Grimmer & Böhnke (1978)
3) Alsberg *et al.* (1982)
4) Hagemann *et al.* (1982)
5) Grimmer & Hildebrandt (1975)
6) µg PAH/g Extract
7) Level relative to B[a]P
8) µg PAH/ Europa-test

Table C.3.2.3.2.1b PAH levels relative to B[a]P in gasoline engine exhaust.

(all values are rounded to two significant figures)

	Model 3		Model 4		Model 5		Model 6		Model 7		Model 8		Model 9		Model 10		Model 11	
Compound	µg/Eur[1]	R.L.[2]	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.
Anthracene	260	11	88	5.3	210	8.8	160	15	250	9.2	140	8.1	370	5.4	290	6.3	130	6.9
Phenanthrene	1000	43	630	38	710	29	600	58	790	29	520	29	1300	19	1300	27	450	24
Fluoranthene	380	17	250	15	460	19	270	27	490	18	310	17	460	6.7	530	11	290	15
Pyrene	420	18	320	20	670	28	370	36	670	25	400	22	530	7.8	630	14	380	20
Benz[a]anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perylene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzo[e]pyrene	28	1.2	18	1.1	30	1.3	12	1.2	33	1.2	21	1.2	65	0.90	49	1.0	20	1.1
Benzo[ghi]perylene	86	3.7	69	4.2	120	5.1	38	3.7	52	1.9	38	2.1	220	3.2	120	2.6	81	4.3
Dibenz[a,h]anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coronene	27	1.2	60	3.7	86	3.5	28	2.7	52	1.9	26	1.5	150	2.3	64	1.4	65	3.4
Indeno[1,2,3-cd]pyrene	20	0.90	15	0.90	24	1.0	8.3	0.80	21	0.80	12	0.60	71	1.0	28	0.60	20	1.1
Anthanthrene	6.5	0.30	9.8	0.60	18	0.70	4.9	0.50	5.9	0.20	4.7	0.30	40	060	18	0.40	15	0.80
Chrysene/Triphenylene	170	7.2	62	3.8	130	5.5	54	5.2	150	5.4	81	4.6	200	2.9	180	3.8	100	5.5
Benzofluoranthenes	34	1.5	19	1.1	34	1.4	13	1.3	32	1.2	20	1.2	100	1.5	68	1.5	24	1.3
Benzo[a]pyrene	23		17		24		10		27		18		68		46		19	

1) µg PAH/Europa-test.
2) Level relative to B[a]P.

Table C.3.2.3.2.1c PAH levels relative to B[a]P in gasoline engine exhaust.

(all values are rounded to two significant figures)

	Model 12		Model 13		Model 14		Model 15		Model 16		Model 17		Model 18		Model 19		Model 20	
Compound	µg/Eur[1]	R.L.[2]	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.	µg/Eur	R.L.
Anthracene	120	2.1	140	7.3	220	4.9	600	5.1	260	15	210	5.6	170	13	150	12	250	5.0
Phenanthrene	720	12	790	42	920	20	2500	21	930	54	760	20	630	46	590	47	880	18
Fluoranthene	550	9.5	360	19	590	13	1200	9.8	320	19	380	10	280	21	240	20	440	8.8
Pyrene	880	15	460	25	930	20	1700	15	350	21	450	12	410	30	340	27	730	14
Benz[a]anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perylene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzo[e]pyrene	55	0.90	23	1.2	40	0.90	92	0.80	22	1.3	36	1.0	18	13	13	1.0	42	0.80
Benzo[ghi]perylene	180	3.0	66	3.5	130	2.8	300	2.5	44	2.6	150	3.9	50	3.6	39	3.1	150	2.9
Dibenz[a,h]anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coronene	110	1.9	34	1.8	78	1.7	160	1.4	17	1.0	98	2.6	34	2.5	32	2.5	80	1.6
Indeno[1,2,3-cd]pyrene	45	0.80	17	0.90	33	0.70	99	0.80	10	0.60	26	0.70	10	0.80	9.3	0.70	40	0.80
Anthanthrene	33	0.60	7.9	0.40	24	0.50	84	0.70	3.6	0.20	32	0.90	7.8	0.60	5.1	0.40	48	0.90
Chrysene/Triphenylene	290	5.0	64	3.4	130	2.9	620	5.2	72	4.2	280	7.5	97	7.1	60	4.8	340	6.7
Benzofluoranthenes	53	0.90	24	1.3	42	0.90	120	1.0	23	1.3	40	1.1	20	1.5	15	1.2	50	1.0
Benzo[a]pyrene	58		19		45		120		17		37		14		12		51	

1) µg PAH/Europa-test.
2) Level relative to B[a]P.

Table C.3.2.3.2.2 Confidence range analysis for gasoline engine exhaust.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	7.0	9.3	5.3	1.8	
Phenanthrene	25	38	17	2.3	
Fluoranthene	7.3	14	4.0	3.5	
Pyrene	9.5	19	4.7	4.0	
Benz[a]anthracene	0.81	-	-	-	
Perylene	0.27	0.60	0.12	4.8	
Benzo[e]pyrene	1.1	1.4	0.79	1.8	
Benzo[ghi]perylene	2.6	3.5	2.0	1.7	
Dibenz[a,h]anthracene	0.072	-	-	-	
Coronene	2.0	2.7	1.4	1.9	
Indeno[1,2,3-cd]pyrene	0.80	1.1	0.60	1.8	
Anthanthrene	0.38	0.55	0.27	2.1	
Chrysene/Triphenylene	3.0	4.6	1.9	2.4	
Benzofluoranthenes	1.1	1.5	0.85	1.7	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.3.3 PAH Profiles from Oil-Fired Power Plants

The PAH content of flue-gas emissions from oil-fired power plants has been presented in section C.3.1.3.3. Only one of these data sets reported B[a]P levels (shown in table C.3.2.3.3.1). Therefore, the means and confidence ranges could not be calculated. These data are, however, included in the comparison of all mixtures (see section C.3.2.5).

Table C.3.2.3.3.1 PAH levels relative to B[a]P in flue-gas emissions from oil-fired power plants (Visit #2).

(all values are rounded to two significant figures)

Compound	μg PAH/m^3	R.L.[1]
Anthracene	-	-
Phenanthrene	-	-
Fluoranthene	0.018	7.5
Pyrene	0.02	9.2
Benz[a]anthracene	-	-
Perylene	0.00080	0.33
Benzo[e]pyrene	0.0057	2.4
Benzo[ghi]perylene	0.00047	0.20
Dibenz[a,h]anthracene	0.00059	0.25
Coronene	0.00030	0.13
Indeno[1,2,3-cd]pyrene	0.00021	0.088
Anthanthrene	-	-
Chrysene/Triphenylene	-	-
Benzofluoranthenes	0.0093	3.9
Benzo[a]pyrene	0.0024	

1) Level relative to B[a]P.

C.3.2.3.4 PAH Profiles in Asphalts

The levels of some PAH identified in roofing and paving asphalts have been presented in section C.3.1.3.4 which indicates that the two types of asphalts should be considered separately. The PAH profiles, the means, the upper and lower 95% confidence limits, and the confidence ranges for roofing and paving asphalts are given in sections C.3.2.3.4.1 and C.3.2.3.4.2, respectively.

Table C.3.2.3.4.1.1 PAH levels relative to B[a]P in roofing asphalts.

(all values are rounded to two significant figures)

	85/40		110/30		95/25		RA#1		RA#2		RA#3	
Compound	µg PAH/g	R.L.[2]	µg PAH/g	R.L.	µg PAH/g	R.L.	µg PAH/g	R.L.	µg PAH/g	R.L.	µg PAH/g	R.L.
Fluoranthene	0.15	0.31	0.40	1.1	0.46	0.96	13	1.6	15	2.8	5.2	2.9
Pyrene	0.17	0.35	0.30	0.86	0.29	0.60	-	-	32	5.9	5.5	3.1
Benz[a]anthracene	0.33	0.67	0.30	0.86	0.23	0.48	380	47	170	31	110	61
Chrysene/Triphenylene	0.90	1.8	1.0	2.9	0.80	1.7	380	47	120	22	45	25
Perylene	0.14	0.29	0.080	0.23	0.20	0.42	10	1.2	3.5	0.65	2.3	1.3
Benzofluoranthenes	0.051	0.10	0.10	0.29	0.04	0.080	2.8	0.35	6.1	1.1	0.29	0.16
Benzo[ghi]perylene	1.3	2.7	1.2	3.4	2.0	4.2	5.5	0.38	4.2	0.78	4.3	2.4
Dibenz[a,h]anthracene	-	-	-	-	-	-	6.7	0.83	-	-	0.39	0.22
Benzo[a]pyrene	0.49		0.35		0.48		8.1		5.4		1.8	

1) µg PAH/g starting material.

2) Level relative to B[a]P.

C.3.2.3.4.1 PAH Profiles in Roofing Asphalts

The PAH profile and confidence range data for roofing asphalt (RA) (tables C.3.2.3.4.1.1 and C.3.2.3.4.1.2) show that of the seven PAH listed, only benz[a]anthracene has a CR value that exceeds 50 (130). The CR values for the remaining six PAH are all less than 25, suggesting that the levels of these compounds, relative to B[a]P, do not vary sufficiently among samples, to alter the estimation of risk. In addition, the levels of B[a]P could be used to estimate the levels of these PAH in other RA mixtures with a reasonable degree of accuracy. In the case of benz[a]anthracene, the number of samples was small. While six samples are acceptable for determination of CR, it is reasonable to assume that a larger number of samples would narrow the confidence range and that the benz[a]anthracene profile would also be reasonably stable.

Table C.3.2.3.4.1.2 Confidence range analysis for roofing asphalts.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	-	-	-	-	
Phenanthrene	-	-	-	-	
Fluoranthene	1.3	3.0	0.54	5.7	
Pyrene	1.3	5.5	0.29	19	
Benz[a]anthracene	5.4	63	0.47	130	*
Perylene	0.55	1.2	0.26	4.6	
Benzo[e]pyrene	-	-	-	-	
Benzo[ghi]perylene	1.9	4.3	0.85	5.1	
Dibenz[a,h]anthracene	-	-	-	-	
Coronene	-	-	-	-	
Indeno[1,2,3-cd]pyrene	-	-	-	-	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	7.8	37	1.6	23	
Benzofluoranthenes	0.23	0.64	0.083	7.7	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.3.4.2 PAH Profiles in Paving Asphalts

The PAH profiles and confidence range data for paving asphalt (PA) (tables C.3.2.3.4.2.1a, C.3.2.3.4.2.1b and C.3.2.3.4.2.2) show that of the seven PAH listed, only benzofluoranthenes has a CR value that exceeds 50 (120). The CR values for the remaining six compounds are below 10, indicating that the levels of these compounds, relative to B[a]P, vary little among PA samples and that this variation is not sufficiently large to alter the estimation of risk. The result also demonstrates that B[a]P could be used to predict the levels of these compounds in PA samples. The lower CR values observed for the PA (all less than 10) as compared to the RA (all less than 25) suggest that the PAH profile is more stable among the PA than among the RA. The higher CR values reported for benzofluoranthenes (120) may be due, in part, to the small number of samples available. The CR values for the other PAH are based on a larger number of samples (ranging from 8 to 13 samples) than the CR value for benzofluoranthenes (only 4 samples). Therefore, it is reasonable to assume that an increase in the sample size in the case of benzofluoranthenes would result in a CR value comparable to those reported for the other PAH. As a result, the PAH profile for all of the PAH listed is expected to be sufficiently stable and not to affect risk estimation.

C.3.2.3.5 PAH Profiles in Petroleum Derived Source Mixtures: Summary

The PAH profiles in various petroleum-derived source mixtures have been examined in sections C.3.2.3.1 through C.3.2.3.4. Only one data set was available for oil-fired power plants. Therefore, confidence ranges could not be calculated for this source. Confidence range analysis for the other sources shows that the CR values for benz[a]anthracene in roofing asphalt and benzofluoranthenes in paving asphalts both exceed 50. For the remaining PAH in asphalts and all of the PAH in diesel and gasoline engine exhausts, CR values do not exceed 50 and many are below 10. Therefore, on a weight of evidence basis, the PAH profiles from petroleum-derived source mixtures could be considered to be constant within the established limit.

Table C.3.2.3.4.2.1a PAH levels relative to B[a]P in paving asphalts.

(all values are rounded to two significant figures)

	A		B		C		D		E		F		G	
Compound	**µg/g[1]**	**R.L.[2]**	**µg/g**	**R.L.**	**µg/g**	**R.L.**	**µg/g**	**R.L.**	**µg/g**	**R.L.**	**µg/g**	**R.L.**	**µg/g**	**R.L.**
Fluoranthene	-	-	-	-	2.0	1.5	-	-	-	-	5.0	0.19	-	-
Pyrene	0.60	1.2	1.8	1.1	4.0	3.1	8.3	3.3	0.90	0.56	38	1.4	0.30	3.0
Benz[a]anthracene	0.25	0.5	2.1	1.2	1.1	0.85	0.70	0.28	0.90	0.56	35	1.3	0.20	2.0
Chrysene/Triphenylene	0.20	0.40	8.9	5.2	2.3	1.8	3.9	1.6	3.2	2.0	34	1.3	0.70	7.0
Perylene	-	-	39	23	2.2	1.7	6.1	2.4	2.9	1.8	3.0	0.11	0.10	1.0
Benzofluoranthenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzo[ghi]perylene	2.1	4.2	4.6	2.7	1.0	0.77	1.7	0.68	2.7	1.7	15	0.56	0.60	6.0
Dibenz[a,h]anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzo[a]pyrene	0.50		1.7		1.3		2.5		1.6		27		0.1	

1) µg PAH/g starting material. 2) Level relative to B[a]P.

Table C.3.2.3.4.2.1b PAH levels relative to B[a]P in paving asphalts.

(all values are rounded to two significant figures)

	I		J		K		L		PA#1		PA#2	
Compound	µg/g[1]	R.L.[2]	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.	µg/g	R.L.
Fluoranthene	0.41	1.9	0.72	0.40	0.46	0.50	0.39	0.35	3.3	2.5	2.7	2.1
Pyrene	0.26	1.2	1.5	0.83	1.0	1.1	1.1	1.0	10	7.7	9.5	7.3
Benz[a]anthracene	0.14	0.64	1.1	0.61	0.89	0.97	0.63	0.57	100	77	84	65
Chrysene/Triphenylene	0.47	2.1	1.5	0.83	3.3	3.6	3.9	3.6	85	65	79	61
Perylene	0.044	0.20	3.3	1.8	0.69	0.75	0.25	0.23	1.7	1.3	1.4	1.1
Benzofluoranthenes	0.24	0.11	0.19	0.11	-	-	-	-	2.0	1.5	1.8	1.4
Benzo[ghi]perylene	1.7	7.7	4.2	2.3	2.3	2.5	2.7	2.5	1.6	1.2	0.92	0.71
Dibenz[a,h]anthracene	-	-	-	-	-	-	-	-	3.7	2.9	5.8	4.5
Benzo[a]pyrene	0.22		1.8		0.92		1.1		1.3		1.3	

1) µg PAH/g starting material.

2) Level relative to B[a]P.

Table C.3.2.3.4.2.2 Confidence range analysis for paving asphalts.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	-	-	-	-	
Phenanthrene	-	-	-	-	
Fluoranthene	0.82	1.9	0.36	5.2	
Pyrene	1.8	2.9	1.1	2.7	
Benz[a]anthracene	1.5	4.4	0.52	8.5	
Perylene	1.0	2.5	0.43	5.9	
Benzo[e]pyrene	-	-	-	-	
Benzo[ghi]perylene	1.9	3.1	1.1	2.8	
Dibenz[a,h]anthracene	-	-	-	-	
Coronene	-	-	-	-	
Indeno[1,2,3-cd]pyrene	-	-	-	-	
Anthanthrene	-	-	-	-	
Chrysene/Triphenylene	3.4	8.5	1.4	6.1	
Benzofluoranthenes	0.40	4.3	0.37	120	*

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.4 PAH Profiles in Other Source Mixtures

The only source mixture considered in section C.3.1.4 is cigarette smoke condensate. The relative levels of PAH in cigarette smoke condensate are discussed in section C.3.2.4.1.

C.3.2.4.1 PAH Profiles in Cigarette Smoke Condensate

The levels of PAH found in cigarette smoke condensate are reported in section C.3.1.4.1. In order to calculate relative levels, the ranges shown in table C.3.1.4.1.1 were divided into two groups identified as "*Low*" and "*High*" which represent the lower and upper bounds of the reported ranges. These levels and the levels relative to B[a]P are reported in table C.3.2.4.1.1. As only two points were available for each PAH, it was not possible to calculate the upper and lower 95% confidence limits, as well as the confidence range. However, the geometric mean of the relative levels for each of the PAH listed was determined. These data were used in the comparison of all mixtures (see section C.3.2.5).

Table C.3.2.4.1.1 PAH levels relative to B[a]P in cigarette smoke condensate.

(all values are rounded to two significant figures)

	Low		High		
Compound	**µg/100 c[1]**	**R.L.[2]**	**µg/100 c**	**R.L.**	**Geo Mean**
Anthracene	2.3	4.6	24	3.1	3.8
Phenanthrene	8.5	17	62	7.9	12
Fluoranthene	1.0	2.0	27	3.5	2.6
Pyrene	5.0	10	27	3.5	5.9
Benz[a]anthracene	0.40	0.80	7.6	0.97	0.88
Perylene	0.30	0.60	0.56	0.072	0.21
Benzo[e]pyrene	0.20	0.40	2.5	0.32	0.36
Benzo[ghi]perylene	0.30	0.60	3.9	0.50	0.55
Dibenz[a,h]anthracene	0.40	0.80	-	-	0.80
Coronene	0.10	0.20	-	-	0.20
Indeno[1,2,3-cd]pyrene	0.40	0.80	20	2.6	1.4
Anthanthrene	0.20	0.40	2.2	0.28	0.33
Chrysene/Triphenylene	0.60	1.2	9.6	1.2	1.2
Benzofluoranthenes	1.6	3.2	5.5	0.71	1.5
Benzo[a]pyrene	0.50		7.8		

1) µg PAH/100 cigarettes. 2) Level relative to B[a]P.

Table C.3.2.5.1 PAH levels relative to B[a]P: all mixtures.

(all values are rounded to two significant figures)

Compound	C.O.	LTCT	HTCT	I&S	C.P.	C.S.	O.W.	W.S.	D.E.	G.E.	O.P.	RA	PA	CSC
Anthracene	2	7.5	3.6	2.3	4.9	-	-	3	-	7	-	-	-	3.8
Phenanthrene	6.7	200	10	9.6	22	-	-	15	-	25	-	-	-	12
Fluoranthene	4	2.3	5.7	3.4	18	24	3.5	5.5	23	7.3	7.5	1.3	0.82	2.6
Pyrene	3	1.4	6.7	3.6	5.2	3.8	4.1	5.5	24	9.5	9.2	1.3	1.8	5.9
Benz[a]anthracene	0.91	1.8	-	1.4	1.8	6.4	0.63	1.2	3.6	-	-	5.4	1.5	0.88
Perylene	0.21	1.4	0.64	0.45	-	2.1	0.83	0.19	-	0.27	0.33	0.55	1.0	0.21
Benzo[e]pyrene	0.56	-	0.89	0.1	0.87	13	1.9	0.65	2	1.1	2.4	-	-	0.36
Benzo[ghi]perylene	0.42	-	-	1.5	-	3.4	0.44	0.71	1.2	2.6	0.2	1.9	1.9	0.55
Dibenz[a,h]anthracene	-	-	-	0.43	-	2.1	0.052	-	0.2	-	0.25	-	-	0.80
Coronene	0.18	-	-	0.45	-	1.3	0.035	-	1.2	2	0.13	-	-	0.2
Indeno[1,2,3-cd]pyrene	-	-	-	1.7	-	-	0.48	0.64	0.16	0.8	0.09	-	-	1.4
Anthanthrene	0.32	-	-	1.4	-	-	-	-	-	0.38	-	-	-	0.33
Chrysene/Triphenylene	1.5	1.1	2	1.7	1.7	10	1.1	1.4	4.1	3	-	7.8	3.4	1.2
Benzofluoranthenes	0.68	1.8	-	1.9	2.6	11	1.2	1.5	5.1	1.1	3.9	0.23	0.40	1.5

1) C.O. = Coke ovens
2) LTCT = Low temperature coal tar
3) HTCT = High temperature coal tar
4) I&S = Iron & steel foundry
5) C.P. = Coal fired power plants
6) C.S. = Coal stoves
7) O.W. = Open and fireplace burning
8) W.S. = Wood stoves
9) D.E. = Diesel emissions
10) G.E. = Gasoline emissions
11) O.P. = Oil fired power plants
12) RA = Roofing asphalt
13) PA = Paving asphalt
14) CSC = Cigarette smoke condensate

C.3.2.5 Comparison of PAH Profiles in All Mixtures

Sections C.3.2.1 through C.3.2.4 examine the levels of individual PAH relative to B[a]P and the stability of the PAH profiles for a number of source mixtures. The data show that, for most source mixtures, the PAH profiles are relatively constant from sample to sample and that any variation that does occur is usually small and not likely to alter the estimations of risk. Section C.3.2.5 examines the variation in PAH profiles among source mixtures to determine if the PAH profiles are the same for all source mixtures examined or if source mixtures must be considered individually. To do this, the mean values calculated for all PAH in all source mixture were used (see table C.3.2.5.1). The mean, upper and lower 95% confidence limit, and confidence range were then calculated for each PAH considered (see table C.3.2.5.2). The results indicate that the confidence range is below 15.0 for all fourteen PAH, suggesting that the PAH profiles do not vary significantly among source mixtures. Any variation, if occurs, is unlikely to be of sufficient magnitude to alter the estimation of risk.

Table C.3.2.5.2 Confidence range analysis for comparison of all mixtures.

(all values are rounded to two significant figures)

Compound	Mean	Upper 0.95	Lower 0.95	CR[1]	Exceeds[2]
Anthracene	3.9	5.8	2.6	9.7	
Phenanthrene	18	44	7.6	2.2	
Fluoranthene	4.9	8.8	2.8	2.7	
Pyrene	4.5	7.1	2.8	5.2	
Benz[a]anthracene	1.8	2.9	1.1	3.3	
Perylene	0.51	0.84	0.31	3.5	
Benzo[e]pyrene	1.0	2.3	0.45	2.4	
Benzo[ghi]perylene	0.98	1.8	0.54	10	
Dibenz[a,h]anthracene	0.35	1.3	0.093	14	
Coronene	0.35	1.1	0.11	3.2	
Indeno[1,2,3-cd]pyrene	0.51	1.4	0.19	7.46	
Anthanthrene	0.49	1.5	0.16	2.7	
Chrysene/Triphenylene	2.3	3.6	1.5	5.9	
Benzofluoranthenes	1.6	2.9	0.84	2.5	

1) Confidence range. 2) CR exceeds 50 (see section C.3.2).

C.3.2.6 Summary

Section C.3.2 attempts to address objectives 4 through 7 outlined in the introduction (see section C.1). These objectives are listed again below.

Objective 4: Determine the similarities or differences which exist in the PAH profiles among mixtures from the same type of source and among mixtures from different sources.

Objective 5: Determine the effect changes in operating conditions has on the PAH profiles of mixtures emitted from a single source.

Objective 6: Determine the effect changes in fuel type has on the PAH profiles of mixtures emitted from a single source.

Objective 7: Determine the similarities and/or differences in PAH profiles which exist among mixtures from all of the sources considered.

The conclusions arising from each objective are discussed below.

Objective 4:

The stability of PAH profiles among mixtures from the same source has been examined for all the source mixtures considered with the exception of the iron and steel foundry (section C.3.2.1.3) and oil-fired power plant (section C.3.2.3.3) mixtures. For these sources, only single data set was available and therefore could not be used for analysis. These data, were however, included in the comparison of all mixtures (section C.3.2.5).

As discussed in the introduction to section C.3.2, it was decided that any compound for which the confidence range (CR) was less than 50 would be considered to have a stable profile relative to B[a]P (i.e. the level of the compound would not vary sufficiently to have an effect on the estimation of risk). The confidence range calculated for all PAH considered in the emissions from coke oven (section C.3.2.1.1), wood stoves (section C.3.2.2.2), diesel exhaust (section C.3.2.3.1) and gasoline engine exhaust (section C.3.2.3.2) are all below 50. The results indicate that these mixtures could be considered to be stable. Data from coal-stoves show that the CR value for one out of 10 PAH listed exceeds 50 (section C.3.2.1.5). The CR values for the PAH content of both roofing and paving asphalt (section C.3.2.3.4) exceed 50 for only one of the seven compounds considered in each mixture. In the case of open and forest fire emissions, the profiles of two out of 11 PAH (section C.3.2.2.1) have CR values which exceed 50. The finding suggests that the PAH profiles of open and forest fire emissions, while less stable than the profiles of the previous mixtures, could be considered to be reasonably stable. The final two mixtures, emissions from coal-tar (section C.3.2.1.2) and coal-fired power plants (section C.3.2.1.4) have two compounds out of seven and eight PAH, respectively, with CR values that exceed the cutoff value of 50. The profiles of these last two mixtures should be

considered to be less stable than the others. However, the fact that only five or six compounds have CR values below 50 suggests that these mixtures could be considered sufficiently stable that estimates of risk based on these profiles would be unlikely to be affected.

Within a given source, the PAH profiles remain relatively constant among the various mixtures considered. Confidence range values which exceed 50 may be a function of both profile instability and of the small number of samples used to determine the CR value. For mixtures where a large number of samples were available, such as gasoline engines (27), wood stoves (11) and diesel emissions (10), the CR values are generally low and do not exceed 50. CR values tend to exceed 50 only when the sample size is small. However, the data are insufficient to determine if small sample size causes CR values to exceed 50 for some PAH in some mixtures. Nevertheless, for all mixtures considered, the CR values for the majority of the PAH do not exceed 50, suggesting that the PAH profiles for all these mixtures could be considered to be sufficiently stable not to affect the final risk estimate.

Objective 5:

The effect of changes in operating conditions on the PAH profile of a mixture can be determined by considering the data from coal-fired power plants (section C.3.2.1.4), diesel (section C.3.2.3.1) and gasoline engine exhaust emissions (section C.3.2.3.2). For the coal-fired power plants, differences in firing systems and pollution controls (Plant H) do not appear to have a significant effect on the PAH profiles. The CR values for six of the eight PAH are below 50 while those for anthracene and chrysene/triphenylene exceed 50, suggesting that differences in the operating conditions may have an effect on the relative levels of these two compounds. However, it should be noted that the fuel, the sampling techniques and the sample size may also play a role in effecting the levels of these PAH. The diesel and gasoline engine exhaust data show that differences in engines and running cycles have little effect on the PAH profiles. The CR values for all PAH in both mixtures do not exceed 50. The finding suggests that difference in operating conditions has little effect on the PAH profiles for a given source mixture.

The coal-fired power plant data show some indication that operating conditions may have an effect on the levels of some PAH. While the available data are limited to fully evaluate this effect, the majority of the data which could be used suggest that the effect on the PAH profiles is minimal. In addition, the effect is unlikely to be sufficiently large to alter the estimations of risk.

Objective 6:

The effect of fuel on the PAH profiles of a given source mixture is best studied by examining the emissions from burning different coal fuels in a single type of stove (see section C.3.2.1.53.2.1.5), and from wood-stoves (see section C.3.2.2.2). In the case of coal stoves, the CR value exceeds 50

for only one of ten PAH emitted. For wood stoves, the CR values for all 11 PAH considered are below 50 and many are below 10. The results suggest that changes in the type of fuel has little effect on the PAH profile of a given mixture.

Objective 7:

Section C.3.2.5 examines the stability of PAH profiles among all mixtures irrespective of their sources and concludes that the levels of PAH relative to B[a]P remain constant among all mixtures examined. The CR values for all of the 14 compounds are less than 15 and are less than 10 for most PAH. Changes in operating conditions and the type of fuel have little effect on the overall stability of the PAH profile. In addition, any changes in relative levels which do occur are unlikely to be of sufficient magnitude to affect the estimation of risk.

Summary:

The data presented show that the PAH profiles of mixtures from the same source remain relatively constant. PAH profiles are also stable among mixtures from different sources. Further, the effects of operating conditions and of fuel type are shown to be minimal. Based on this information, one can conclude that the PAH profile is constant within the defined limit and is independent of the source, the operating conditions or the fuel type.

C.4 Summary of Conclusions

The purpose of appendix C is to validate the assumptions on which the relative potency approach is based. The assumptions are the following. 1. The total amount of PAH remains constant for a given mixture regardless of the fuel or combustion/operating conditions (Albert *et al.* 1983). 2. The levels of individual PAH, relative to a fixed marker (PAH profile), also remains constant. In order to validate these assumptions, a number of objectives were addressed. These are listed below. Objective 1 through 3 test the validity of the first assumption and examine the total PAH content of mixtures from various sources (see section C.3.1). The conclusions drawn from objectives 1 through 3 are discussed in detail in section C.3.1.6. Objectives 4 through 7 test the validity of the second assumption and are addressed in section C.3.2. The conclusions drawn from these objectives are discussed in detail in section C.3.2.6. The current section provides only a summary of the work and draws conclusions regarding the validity of the two assumptions being tested.

Objectives Addressed in Section C.3

Objective 1: Determine the differences in total PAH content which exist among mixtures from the same type of source and among mixtures from different sources.

Objective 2: Determine the effect changes in operating conditions have on the levels of PAH emitted from a source.

Objective 3: Determine the effect changes in fuel have on the levels of PAH emitted from a source.

Objective 4: Determine the similarities or differences which exist in the PAH profiles among mixtures from the same type of source and among mixtures from different sources.

Objective 5: Determine the effect changes in operating conditions have on the PAH profiles of mixtures emitted from a single source.

Objective 6: Determine the effect changes in fuel type have on the PAH profiles of mixtures emitted from a single source.

Objective 7: Determine the similarities and/or differences in PAH profiles which exist among mixtures from all the sources considered.

Assumption 1: The total amount of PAH in a mixture remains constant regardless of the fuel or combustion conditions.

Section C.3.1 examines the similarities and differences in the levels of PAH among mixtures from the same type of source and among mixtures from different sources. The data show that for mixtures from the same type of source, the levels of individual PAH can vary by over two orders of magnitude (see section C.3.1.6). Among mixtures from different sources, variation can be over six orders of magnitude (see table C.3.1.5.2 and section C.3.1.6). It should be noted that differences in reporting formats make quantitative comparison difficult and that the variation in PAH level could be larger than what is reported here.

Objectives 2 and 3 examine the effect of fuel and operating conditions (combustion conditions) on the PAH content of source mixtures (see section C.3.1.6). The data presented show that changes in both of these factors can alter the levels of PAH emitted from a source. The magnitude of the effect depends on the source being considered.

Based on the data presented in section C.3.1 and summarized above, one can conclude that the assumption that the PAH content of a mixture remains constant regardless of the fuel and/or operating conditions is not valid.

Assumption 2: The PAH profiles of a mixture remain constant.

Section C.3.2 examines the similarities and differences in PAH profiles among mixtures from the same source and among mixtures from different sources. The effect of altered fuel or operating conditions have also been examined. For all mixtures considered, the levels of most of the PAH examined remain

constant within the limit established in section C.3.2. Changes in fuel or operating conditions have little effect on the PAH profiles within a given mixture. In addition, when the PAH profiles are compared among all the mixtures, the PAH profile is found to remain constant within the established limit (see section C.3.2.5).

Based on the data presented, one can conclude that the levels of individual PAH relative to a fixed marker (B[a]P) remains constant and the changes in fuel and/or operating conditions do not significantly alter these profiles. Therefore, the second assumption can be considered valid.

Summary:

The data presented in this appendix show that the assumption, that the PAH content remains constant in a given mixture regardless of changes in fuel or operating conditions, is not valid. The assumption that PAH profiles remain constant is found to be valid not only for mixtures from a single type of source, but also for mixtures from differing sources. Therefore, the *Relative Potency Approach* does not hold when total PAH levels are considered. However, it appears to hold when PAH profiles, instead of total PAH content, are considered .

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APPENDIX D

METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS

by Bryan Leece

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APPENDIX D METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS

D.1 Introduction

D.1.1 Document Preface

This appendix is intended to meet a number of objectives in the development of the Scientific Criteria Document for PAH. It is intended to provide supporting information for a number of issues addressed in the main document and in other appendices. The objectives of the metabolism section are outlined below.

Table D.1-1 Objectives of Appendix D: Metabolism of PAH

Title	Objective
Grouping PAH:	Determine if PAH can be grouped based on their metabolic behaviour.
Species Differences:	Determine the differences in PAH metabolism between species.
Route of Administration:	Determine the differences in metabolism of a compound when it is administered by different orally, dermally or by inhalation.

In order to meet the objectives outlined above, a number of questions must be addressed. The questions associated with each objective are listed below.

Grouping PAH:

1) Are all PAH metabolized by the same enzymes?

2) Are similar metabolites formed for all PAH?

3) What structural features govern the metabolic processing of PAH?

Species Differences:

1) Are the enzyme activities the same in different species?

2) Are the metabolite profiles for a given PAH the same in different species?

3) For a given PAH are the PAH-DNA adduct profiles the same in different species?

Route of Administration:

1) Does the PAH metabolite profile in a given tissue change when a compound is administered orally, dermally or by inhalation?

2) Do differences in enzyme profiles exist between various tissues within a single species?

3) Does the route of administration affect the quantity and site of DNA adduct formation?

From the questions outlined above, it is clear that a knowledge of PAH metabolism, the enzymes involved and the products formed is essential to meet the overall objectives of the appendix. Further, knowledge of metabolism enzymes and adduct formation is common to all objectives. The following sections address PAH metabolism (Section D.1 Basic Review), the enzymes involved (Sections D.2 and D.3) and the formation of DNA adducts (Section D.4). The final section of this document (Section D.5: Summary of Conclusions) uses the information assembled in Sections D.1 through D.4 to answer each of the question outlined above. The reader is cautioned than, unlike other sections of the scientific criteria document, this appendix has not had the benefit of peer review. Therefore caution must be exercised when evaluating the conclusions drawn in this appendix.

A Note Regarding Referencing:

The referencing scheme used in this appendix differs from that used in the main document and the other appendices. A reference code has been generated for each of the referenced cited in this appendix. This appendix uses a six character code for example; JRET96. The first letter is either a "B" which indicates a book or a "J" which indicates a journal. The next three characters represent the first three letters of the first authors name. The final two number indicate the year of publication. In cases where the authors last name has only two letters, a "*" is used in place of a third letter. Where more than one reference is used from a given author in a given year, the reference codes are amended with a final letter eg "a, b, etc.....". For each reference, the reference code is listed beside the full citation in the reference section of the report.

D.1.1 Introduction

Most polynuclear aromatic hydrocarbons (PAH) are highly hydrophobic compounds which must be metabolically modified before they are sufficiently water soluble to be excreted (JGEL80). The metabolism of PAH can be broken down into two discrete and sequential stages, identified as Phase I and Phase II. Both phases are controlled by a number of enzymes systems and both produce a multitude of products.

The metabolic reactions of Phase I increase the water solubility of a parent compound usually through an oxidation process which adds one or more hydroxyl groups to the core. These initial reactions are regulated by isozymes of cytochrome P-450 and by epoxide hydrolase (EH). Although the formation of hydroxylated products represents a major route of PAH parent metabolism, alkylation reactions are also known to occur (BFLE88,BFLE85,BFLE90). A parent PAH compound entering Phase I metabolism, exits with a slightly increase water solubility which is frequently insufficient to insure its removal. Consequently, the compound will either undergo further Phase I metabolic modification or will be passed on to Phase II for processing.

Phase II reactions increase the water solubility of metabolites by conjugating these molecules with highly hydrophilic moieties such as glutathione and glucuronic and sulphuric acids (JGRO86, BCOO83, BNEM81). In order for the conjugation reactions to occur, the substrate molecule must contain either a sufficiently reactive center in the core or a reactive side group capable of accepting the hydrophilic moiety from the transferring enzyme (BNEM81). In general, PAH do not contain the requisite reactive centers and must pass through Phase I metabolism before they can be conjugated and removed. Some substituted PAH, particularly amino, nitro and hydroxy-substituted PAH, are able to undergo Phase II conjugation without prior Phase I activation. While these compound are able to sidestep Phase I, many still pass through Phase I before being passed to Phase II.

The metabolic transformation of PAH serves as the initial step in the generation of reactive intermediates which have the capacity to react with a variety of subcellular components. These interactions have the potential to lead to altered cell function (BEST78). Although PAH vary greatly in size, ring configuration and substitution patterns, most undergo similar reactions in Phase I and Phase II metabolism and produce similar kinds of products. Differences arise in the location of substitution and the reactivities of intermediate metabolites formed during the process. The activation of classical PAH to carcinogenic metabolites involves the formation of dihydrodiol-epoxides (BEST78). The ultimate carcinogen however, is a carbonium ion formed upon opening the epoxide ring of a dihydrodiol-epoxide (BSIM81). Stable carbonium ions are more readily formed from dihydrodiol-epoxides where the epoxide is adjacent to a bay region (vicinal dihydrodiol-epoxide) rather than in non-bay regions (BSIM81, BJER77, BJER78, JLEH82). Substituted PAH are subject to the same ring oxidation reactions as classical PAH, and can be converted to active vicinal dihydrodiol-epoxides. Many substituted PAH (Amino, Methyl and Nitro) can also be activated through metabolic processes which involve only the substituent group.

D.1.2 Phase I Metabolism

D.1.2.1 Primary Phase I Metabolism

Oxidation of the PAH core is the first step in the generation of PAH metabolites. Cytochrome P-450 assisted addition of an atom of oxygen across a double bond produces a primary epoxide (see table D.1.1-1). While the formation of epoxides by cytochrome P-450 is common, the enzyme is also capable of carrying out single carbon oxidations at centers where there is sufficient electron density (C6 of B[a]P for example). Oxidation at a single carbon usually results in the production phenol or quinone derivatives. For example, oxidation of benzo[a]pyrene at C6 yields an unstable 6-hydroxyB[a]P derivative which rapidly rearranges to one of three possible quinones 1,6-, 3,6- and 6,12-B[a]Pquinones (BCOO83). Dihydrodiols, phenols and quinones each account for approximately 30 percent of the B[a]P metabolites formed (BEST78). Although oxidation by cytochrome P-450 is the major route of metabolism for unsubstituted PAH, these compounds are also alkylated by S-adenosylmethionine transferase (SAM T) reactions (BFLE88, BFLE85, BFLE90). Alkylated products are processed in the same manner as naturally occurring alkylated PAH.

D.1.2.2 Secondary Phase I Metabolism

The epoxide ring formed by cytochrome P-450 is open to a number of processes, each yielding different products (see table D.1.1-2). Epoxides can rearrange spontaneously to form phenolic metabolites (JGRO86, BCOO83, BSIM83. Epoxide hydrolase opens the ring structure by adding a molecule of water across the ring to produce a dihydrodiol. Glutathione Transferases (GST) can act directly upon simple epoxides producing conjugates which are excreted (JGRO86). Epoxide reductase reduces the epoxide to yield the parent PAH and water (BCOO83, BYUZ78).

Table D.1.1-1: Primary Metabolites of Unsubstituted PAH Arising From Hepatic Phase I Metabolism

Substrate	Product	Enzyme	Reference
Unsubstituted PAH	Primary Epoxide	Cytochrome P-450s*	BEST78, BSIM81, BOSB87, BYAN78
Unsubstituted PAH	Phenols	Cytochrome P-450s	BYUZ78
Unsubstituted PAH	Quinones	Cytochrome P-450s	
Unsubstituted PAH	Methylated PAH	SAM**	BFLE88, BFLE85

* Cytochrome P-450s indicates more than one isozyme may be responsible for the formation of the product.

** S-Adenosylmethionine Transferases

Table D.1.1-2: Secondary Metabolites of Hepatic Phase I Metabolism

Substrate	Product	Enzyme	Reference
Primary Epoxide	Dihydrodiol	Epoxide Hydrolase	BCOO83, BYUZ78, BOSB87
Primary Epoxide	Dihydrodiol	Non-Enymatic	BYUZ78
Primary Epoxide	Phenols	Non-Enzymatic	BYUZ78
Primary Epoxide	Parent PAH	Epoxide Reductase	BCOO83, BYUZ78
Methylated PAH	Hydroxymethyl PAH	Cytochrome P-450s	BFLE85
Phenols	Phenol-Epoxides	Cytochrome P-450s	BCOO83
Phenols	Quinones	Cytochrome P-450s	BCOO83
Phenol-Epoxides	Triols	Epoxide Hydrolase	BCOO83
Dihydrodiols	Dihydrodiol-Epoxides	Cytochrome P-450s	BYUZ78
Dihydrodiol-Epoxide	Triols	Non-Enzymatic	BCOO83
Dihydrodiol-Epoxide	Tetrahydrotetrols	Non-Enzymatic	BCOO83
Dihydrodiol-Epoxide	DNA ADDUCT*	Non-Enzymatic	JGRO86, BCOO83, BYUZ78, BOSB87
Triol	Triol-Epoxide	Cytochrome P-450s	BCOO83

* Vicinal Dihydrodiols are capable of forming stable carbonium ions which are thought to be the ultimate carcinogens.

D.1.2.3 Secondary Phase I Metabolism of Dihydrodiols

D.1.2.3.1 Formation of Dihydrodiol-Epoxides

The formation of dihydrodiols from primary epoxides is an essential step in the formation of vicinal dihydrodiol-epoxides (see table D.1.1-2). The rate of dihydrodiol formation is dependent upon EH which is capable of hydrating both K and Bay-region epoxides (BSIM81, BYAN78). In the absence of glutathione transferase, a high cytochrome P-450 to EH ratio favours phenol formation, while a high EH to cytochrome P-450 ratio favours the formation of dihydrodiols (BYAN78). In the absence of glutathione transferase and EH, very little dihydrodiol is formed (BSIM81). Some epoxides of B[a]P undergo spontaneous hydration to form dihydrodiols, but this is thought to be a very minor route (BYUZ78). The formation of non-K-region dihydrodiols is significant because these metabolites contain isolated olefinic double bonds in positions which allow the easy formation of secondary metabolites (BSIM81). While the vicinal double bond of a non-K-region dihydrodiol is the most olefinic double bond in the PAH, it may not be the major site of further metabolism (BSIM81, BYAN78). Diol conformation and the regioselectivity of cytochrome P-450 appear to be the major determining factors in subsequent metabolic processing (BSIM81, BYAN78). Dihydrodiols which adopt a quasi-diequatorial conformation appear to be preferentially metabolized to vicinal dihydrodiol-epoxides. Dihydrodiols which adopt a quasi-diaxial conformation are generally metabolized at sites removed from the olefinic double bond (BSIM81). For example, B[a]P-7,8-dihydrodiol adopts a quasi-diequatorial conformation and is converted to a B[a]P-7,8-dihydrodiol-9,10-epoxide while B[a]P-9,10-dihydrodiol adopts a quasi-diaxial conformation and is metabolized at sites removed from the vicinal double bond (principally C1 and C3) (BSIM81, BYAN78). Dihydrodiols represent approximately 30 percent of the metabolites formed from B[a]P. Of this, the predominant form is B[a]P-9,10-dihydrodiol (BEST78).

In general, the activation of PAH to carcinogenic metabolites involves the formation of vicinal diol-epoxides. The ultimate carcinogen however, is a carbonium ion. The carbonium ion is formed by the opening of the epoxide ring. Vicinal diol-epoxides may be more effective as reactive intermediates than primary epoxides because they may not be suitable substrates for epoxide hydrolase and glutathione transferase (BCOO83).

D.1.2.3.2 Formation of Triols and Tetrols

Trihydroxy (triol) metabolites of PAH can be formed by two distinct mechanisms (see table D.1.1-2). The first involves the oxidation of phenolic metabolites and the second the hydrolysis of dihydrodiol-epoxides. The hydrolysis of dihydrodiol-epoxides to triol products is a non-enzymatic reduction which requires NADPH (BCOO83, BEST78).

Tetrahydrotetrols are formed by the addition of H_2O across the epoxide of a dihydrodiol-epoxide compound (see table D.1.1-2)(BCOO83, BEST78). Because the addition can be either *cis* or *trans*, two distinct compounds can be produced from a single dihydrodiol-epoxide. A *trans* addition of H_2O across the 9,10-epoxide of anti-7,8-dihydrodiol-9,10-epoxideB[a]P will produce (7,10/8,9)-tetrahydrotetrahydroxyB[a]P, while the cis addition of H_2O yields (7/8,9,10)tetrahydrotetrahydroxyB[a]P (BCOO83, BEST78). This hydrolysis can be catalyzed by a number of acids including DNA (BCOO83, BEST78).

D.1.2.4 Secondary Phase I Metabolism of Phenols

Cytochrome P-450 is capable of further oxidizing phenolic compounds to phenolic epoxides and quinones (see table D.1.1-2)(BCOO83). Epoxides arising from this mechanism are formed at sites removed from the original site of oxidation. Subsequent hydrolysis of the epoxide by EH yields a trihydroxy (triol) derivative (BCOO83). For example, both 3-hydroxy and 9-hydroxy are metabolized to the corresponding B[a]P-4,5-epoxide. Hydrolysis of this epoxide produces 3,4,5-trihydroxy and 4,5,9-trihydroxyB[a]P respectively (BCOO83). Quinones are formed from phenolic metabolites if the initial hydroxyl group is in an appropriate location. For example, 3-hydroxyB[a]P can be converted to B[a]P-3,6-quinone (BCOO83).

D.1.3 Phase II Metabolism

There are a number of enzymes, both membranous and cytosolic, which are involved in the Phase II metabolic processing of PAH. While Glutathione-transferase, Glucuronyl-transferase and Sulfo-transferase are the major enzymes involved in the conjugation of metabolites of classical, substituted and heterocyclic PAH, other enzymes are involved in minor pathways. The enzymes responsible for Phase II metabolism, their substrates and products are listed in table D.1.2-1. The relative importance of any given enzyme pathway depends upon the type of PAH being metabolized. For classical PAH, the myriad metabolic products formed during Phase I metabolism means that most Phase II enzyme pathways will be responsible for the removal of a significant proportion of the metabolites formed. Substituted PAH frequently contain substituent groups which are sufficiently reactive to function as substrates for Phase II enzymes without prior Phase I activation. For these compounds the relative importance of the individual Phase II enzymes differs from that of the classical PAH.

D.1.4 Metabolism of Substituted PAH

Substituted PAH undergo many of the same reactions as classical PAH but have the added potential to be metabolized at the substituent group (see table D.1.3-1). Frequently, it is this side group metabolism which generates the final reactive product. Amino and nitro substituted PAH can be converted to aminoacyl intermediates which are capable of interacting with cellular macromolecules. The metabolism of each substituent group will be dealt with separately.

Table D.1.2-1: Products of Hepatic Phase II Metabolism

Substrate	Product	Enzyme	Reference
Primary Epoxides	Mercapturic Acids[1]	GST[2]	JGRO86, BNEM81, BSIM81, BGUE81
Phenols	Sulphate Esters	ST[2]	JGRO86, BNEM81,
Phenols	Glycosides[3]	UDP-GT[4]	BCOO83, BNEM81,
Quinones	Sulphate Esters	ST	JGRO86, BNEM81,
Quinones	Glycosides	UDP-GT	BCOO83, BNEM81
Dihydrodiols	Sulphate Esters	ST	JGRO86, BNEM81,
Dihydrodiols	Glycosides	UDP-GT	BCOO83, BNEM81
Dihydrodiols	Catechols	Dihydrodiol Dehydrogenase	BCOO83
Triol			
Triol-Epoxides	Mercapturic Acid	GST	JGRO86, BNEM81, BSIM81, BGUE81
Tetrahydrotetrols	Glycosides	UDP-GT	BCOO83, BOSB87

1 Glutathione Transferases produce glutathione conjugates which are further processed to a final mercapturic acid.

2 GST = Glutathione Transferase: ST = Sulpho-Transferases:

3 UDP-Glucuronyl-Transferases produce glucuronic acid conjugates which are further processed to yield glycoside products.

4 Glucuronyl-Transferases

Table D.1.3-1: Metabolites of Substituted PAH.

Substrate	Product	Enzyme	Reference
Nitro Substituted PAH	Amino Substituted PAH	Nitro Reductases	JHOW88
Nitro Substituted PAH	Amino Substituted PAH	Non-Enzymatic	JHOW88
Nitro Substituted PAH	Ring Oxidized PAH	Cytochrome P-450s	JHOW88, JRIC87
Amino Substituted PAH	N-Hydroxylated PAH	Cytochrome P-450s	JFU*82
Amino Substituted PAH	N-Acetylated PAH	??	JFU*82
Amino Substituted PAH	Ring Oxidized PAH	Cytochrome P-450s	JFU*82
Alkyl Substituted PAH	Hydroxyalkyl PAH	Cytochrome P-450s	BFLE85, BHEC88, JCHR86, BSUL92
Alkyl Substituted PAH	Ring Oxidized PAH	Cytochrome P-450s	
Chloro Substituted PAH	Ring Oxidized PAH	Cytochrome P-450s	
Hydroxy Substituted PAH	Ring Oxidized PAH		

D.1.4.1 Metabolism of Methyl-Substituted PAH

Methylated PAH are subject to metabolic attack both on the PAH core and on the methyl substituent. Cytochrome P-450 is able to carry out many of the common ring oxidation reactions producing epoxides which are converted to dihydrodiols, phenols and quinone (BHEC88, JCHR86, JBAY86, JBAO86, BSUL92). Dihydrodiols are further open to conversion to dihydrodiol-epoxides (BHEC88, JCHR86, JBAY86, JBAO86, BSUL92). Dihydrodiols, phenols and quinones are conjugated and excreted (BHEC88).

The location of the methyl substituent has a marked impact on the metabolism of a PAH. For example, the addition of a methyl group to C_5 of chrysene increases the tumorogenicity of the compound compared to the parent by facilitating the formation of a 1,2-dihydrodiol-3,4-epoxide (BSUL92). A methyl substitution at C_7 of B[a]P leads to the formation of a 9,10-dihydrodiol-7-methyl-B[a]P while a methyl substitution at C_{10} produces two epoxides; one at 4,5 and one at 7,8 (BSUL92).

Cytochrome P-450 is able to carry out a single electron oxidation of the methyl group to produce a hydroxymethyl group (BFLE85, BHEC88, JBAO86). This has been proposed as the first step in an alternate activation pathway (BFLE85). Subsequent processing of the hydroxymethyl group by PAPS forms a methyl-sulphate ester. The sulphate moiety is a good leaving group and is easily removed to produce a benzylic carbonium ion (BFLE85, JBAO86). It is this carbonium ion which is thought to be capable of binding to DNA and other cellular macromolecules (BFLE85, JFU*82, JCHR86). The importance of this reaction in the generation of reactive metabolites is however, in question. Studies which have investigated the tumorogenicity of 5- and 6-hydroxymethylchrysenes have found that the 5-hydroxy compound showed tumorogenic potency approximately equal to that of 5-methylchrysene while the 6-hydroxy derivative showed little or no tumorigenic activity (JBAO86).

D.1.4.2 Metabolism of Amino-Substituted PAH

Amino-substituted PAH are subject to ring and N-oxidation reactions (JHOW88, JFU*82). Ring-oxidation reactions generate similar metabolites to those produced from the parent PAH. Metabolic N-oxidation of amino-substituted PAH can follow either of two competing pathways; N-hydroxylation or N-acetylation (JHOW88, JFU*82).

Ring-oxidation of amino-substituted PAH is influenced by the location of the amino group (JHOW88, JFU*82). The normal pattern of oxidation may be altered if the amino group is located at or near a site of normal ring epoxidation. It has been postulated that the increased mutagenic activity in, the Ames test, observed for 4-aminobenzo[a]pyrene compared to benzo[a]pyrene may be due to the presence of the amino group adjacent to a primary site of epoxidation (JHOW88, JFU*82). The blocking of this normal pathway could redirect metabolism to the benzyl ring region of the molecule resulting in an increased formation of the active 7,8-diol-9,10-epoxide B[a]P derivatives (JFU*82). Likewise the observed reduction in Ames test mutagenicity of 6-, and 11-aminobenzo[a]pyrene compared to B[a]P may be attributed to a reduction in ring-oxidation on the benzylic ring of B[a]P as a result of the presence of an amino group adjacent to the site of epoxidation (JHOW88, JFU*82). Cytochrome P-450 oxidation can lead to the formation of ring hydroxylated products in which the hydroxyl group is located adjacent to the amino substitution. The presence of the amino group facilitates a positive charge delocalization over the ring. This allows the enzymatic introduction of a hydroxyl group to take place either on the amino group or on a neighboring ring carbon (JHOW88).

Cytochrome P-450 mediated N-hydroxylation of amino substituted PAH, serves as an alternate activation pathway to ring-oxidation. N-hydroxylated products are proximate carcinogens. Final conversion to ultimate carcinogens requires a variety of post-oxidation enzymes including peroxidases and sulpho-, acyl-, aminoacyl-, and glucuronyl transferases (JHOW88). N-hydroxylated metabolites can also be converted to O-acetyl derivatives through acetyl-CoA dependent reactions. O-acetylated products can be converted into acetoxy esters which are able to bind to DNA (JFU*82). Enzymes responsible for N-acetylation reactions are also able to carry out O-acetylation reactions (JFU*82). The extent to which an aromatic amine is converted to a N-hydroxylated product depends upon the structure of the molecule and the cytochrome P-450 isozymes present (JHOW88, JFU*82).

N-acetylation reactions can be considered as true detoxification pathways. Formation of N-acetylated metabolites does not lead to the subsequent production of reactive metabolites. Further, their formation decreases the amount of aromatic amine which is available for N-hydroxylation (JFU*82).

D.1.4.3 Metabolism of Nitro-Substituted PAH

The metabolism of nitro-substituted PAH has been extensively reviewed by Fu (JFU*90). In mammalian systems, at least five pathways have been shown to lead to the production of potentially reactive metabolites (JHOW88, JRIC87, JFU*90, BHOW85, BHOW88).

i) Nitro-reduction.
ii) Nitro-reduction followed by esterification.
iii) Ring-oxidation.
iv) Ring-oxidation followed by nitro-reduction.
v) Ring-oxidation followed by nitro-reduction and esterification.

Enzymatic reduction of the nitro group is thought to be an important activation pathway (JHOW88). It has been proposed that, the metabolic activation of nitro-substituted PAH involves ring-oxidation followed by nitro-reduction to produce ring oxidized N-hydroxylamino-PAH derivatives as penultimate products (JHOW88). In mammalian systems, these compounds have the potential to undergo further activation to acylated derivatives through acetyl-CoA dependent O-acetyl-transferase mediated reactions (JHOW88).

Cytochrome P-450 is responsible for the ring oxidation of nitro-substituted PAH (JHOW88, JRIC87, JFU*90, BHOW85, BHOW88). Most mono-nitro-substituted PAH are detoxified by ring-oxidations to yield dihydrodiols, phenols, triols and tetrols. These metabolites are further processed by Phase II enzymes before the compounds are finally excreted as glutathione, glucuronide or sulpho-conjugates (BHOW88). Nitro-substitution does not appear to affect stereoselectivity but does alter the regioselectivity of the oxidation reactions (JHOW88). The metabolism of 9-nitroanthrcene to *trans* 3,4-dihydroxy-3,4-dihydro-9-nitroanthracene and 7-nitrobenz[a]anthracene to its corresponding 3,4-dihydrdiol suggest that aromatic ring oxidation is inhibited at positions located *peri* to the nitro group (JHOW88, JRIC87). Formation of a dihydrodiol in positions *peri* to the nitro group, changes the conformation of the dihydrodiol from quasi-diequatorial to quasi-diaxial (JHOW88).

The nitro-reduction of nitro-substituted PAH is controlled by enzymes not normally associated with Phase I metabolism, including; xanthine oxidase and alcohol dehydrogenase (JHOW88, JFU*90). Cytochrome P-450 has been shown to be capable of carrying out some nitro-reduction reactions in *in vitro* systems under anaerobic conditions (JRIC87). Reduction of the nitro substituent is a three step process. Initially, the nitro group is reduced to a nitroso derivative. Subsequent processing further reduces this through an N-hydroxylamino to a final amino-PAH product (JFU*90). Both the nitroso and N-hydroxylamino intermediates are potential electrophiles, but only the N-hydroxylamino appears to bind to DNA (JFU*90). While nitro-substituted PAH might be expected to undergo Phase II reactions without prior Phase I processing, this does not seem to be the case. Most nitro-substituted PAH pass through either ring-oxidation or nitro-reduction before they form conjugates in Phase II. However, some nitro-substituted PAH, (1-nitropyrene, 1-nitrobenzo[a]pyrene and 6-nitrochrysene) have been shown to significantly increase the levels of UDP-glucuronyltransferases and cytosolic glutathione transferases when administered intraperitoneally to rats (JFU*90). Sulphotransferases do not appear to be affected (JFU*90).

The reduction of nitro-substituted PAH can proceed by either enzymatic or non-enzymatic routes. Nitro-PAH can be reduced non-enzymatically by ascorbate or reduced pyridine or flavin nucleotides (JFU*90). Enzymatic reduction of nitro-substituted PAH produces nitrosoarenes, N-hydroxyarylamino-PAH and arylamines. Nitro and nitroxide radicals have also been detected (JHOW88). Nitrosoarenes and H-hydroxyarylamino-PAH can be further reduced enzymatically to arylamine by a number of enzyme systems throughout the cell (JHOW88). Arylamines produced in this manner may be passed on to the enzymes responsible for the metabolic removal of amino-substituted PAH.

D.1.4.4 Metabolism of Hydroxy-Substituted PAH

Hydroxy-substituted PAH are subject to the same reactions as classical PAH. The location of the hydroxy substituent can effect the metabolic profile in that its location can dictate favorable and unfavorable steric processing. Because hydroxy PAH are normal products of classical PAH metabolism, these compounds will be processed in the same way as other phenolic and dihydrodiol PAH metabolites. They will be subject to further phase I metabolism and to phase II conjugation reactions.

D.1.4.5 Metabolism of Halo-Substituted PAH

The metabolism of halo-substituted PAH has not been studied to any great extent. The little information available seems to indicate that the halo substituent does not take an active part in the metabolic process. The location of the substituent can direct metabolic reactions either toward reactive centers or away from these centers depending upon its location on the core molecule. Metabolism of other halo-substituted organic compounds involves the halo group directly. These reactions are usually halo-substitution reaction in which the halo group is removed from the molecule and is replaced either by a hydrogen or a hydroxyl group. These types of reactions do not seem to occur in PAH metabolism.

D.1.5 Metabolism of Heterocyclic PAH

The metabolism of heterocylic PAH parallels that of classical PAH in many ways. Many of the same products are formed (See Table D.1.4-1) However, the presence of heterocylic atoms can have a marked effect on the observed metabolic profiles. Nitrogen heteroatoms are rarely the site of oxidation reaction, while sulphur heteroatoms are the predominant site of oxidation (BLEH88, BJAC88, JBER86). Although nitrogen heteroatoms are not directly involved in oxidation reactions, their presence can direct oxidation reactions at other sites in a PAH molecule (JWOO86, JWOO89). The metabolism of nitrogen and sulphur containing PAH molecules is discussed below.

D.1.5.1 Metabolism of Nitrogen Heterocyclic PAH (Acridines)

Investigations of the metabolism of nitrogen containing heterocyclic PAH have focussed on acridine and its benzo and dibenzo- derivatives (BLEH88, JWOO86, JWOO89, JDUK88). The metabolic transformation of acridines appears to be similar to that of classical PAH. The major metabolites of unsubstituted acridines include epoxides, dihydrodiols, dihydrodiol-epoxides, phenols and *bis*-dihydrodiols (tetrols) (BLEH88). The metabolism of methyl-substituted acridines such as 7-methylbenz[c]acridine also yield hydroxymethyl derivatives (BLEH88). There is little evidence that the nitrogen heteroatom undergoes direct oxidation to any significant degree (BLEH88). Furthermore, the formation of acridone (N=O) metabolites occurs only with acridine (BLEH88).

Studies of the metabolism of benz- and dibenzacridines suggest that the formation of bay-region diol-epoxides are, at best, minor routes (BLEH88). However, rat liver microsomal metabolism of dibenz[a,j]acridine produces extensive amounts of dibenz[a,j]acridine-3,4-dihydrodiol which has a bay region double bond and a K-region epoxide (dibenz[a,j]acridine-5,6-epoxide) (JDUK88). Respectively, these compounds account for 40% and 20% of the metabolites formed (JDUK88). The formation of dibenz[a,j]acridine-3,4-dihydrodiol is of concern because it is the precursor of the putative ultimate carcinogen dibenz[a,j]acridine 3,4-dihydrodiol-1,2-epoxide. The formation of a bay-region diol-epoxide from dibenz[a,j]acridine however is likely to be limited. Studies which have investigated the mutagenicity of dibenz[a,h]acridine and dibenz[c,h]acridine have found that the nitrogen heteroatom plays a role in the stabilization of the carbocation formed upon the opening of an epoxide ring (JWOO86, JWOO89). The formation of a carbocation is favoured when the epoxide ring opening takes place adjacent to the nitrogen heteroatom. Resonance delocalization of the positive charge is directed away form the nitrogen atom in these circumstances. When the epoxide ring is located on the opposite side of the ring from the nitrogen atom, delocalization of the carbocation charge results in an unfavorable charge on the nitrogen atom. This may have a destabilizing effect which would prevent the formation of a bay-region diol-epoxide (JWOO86, JWOO89). This is supported by the low observed activity of dibenz[c,h]acridine-3,4-dihydrodiol, a compound which has a bay region double bond open to diol-epoxide formation (JWOO86).

Phase II metabolism of nitrogen heterocylic PAH can be expected to follow the same pathways as classical PAH, resulting in glutathione, glucuronide and sulpho-conjugate products.

D.1.5.2 Metabolism of Sulphur Heterocyclic PAH (Thiophenes)

The metabolism of sulphur containing PAH (thiophenes) is dependent upon the susceptibility of the sulphur atom to oxidation. Oxidation of the sulphur atom produces sulphoxides and sulphones (BJAC88, JBER86). Ring oxidation reactions produce epoxides, dihydrodiols, dihydrodiol-epoxides, phenols and quinones. Sulphone-phenolic products have also been identified, but it is unclear if the phenol formation precedes or follows the oxidation of the sulphur atom, or if the order of oxidation is important (BJAC88).

Investigations of thiophenes using rat liver microsomal preparations from control and treated animals have shown that the cytochrome P-450 system is able to produce many of the observed metabolites (BJAC88, JBER86). Microsomes from untreated rat liver can metabolize thiophene PAH analogues primarily through sulphur oxidation reactions to produce sulphoxides and sulphones (BJAC88). Induction of cytochrome P-450b with compounds like DDT enhances sulphone formation. Induction of cytochrome P-450c by benzo[k]fluoranthene treatment increase ring oxidation reactions leading to the formation of dihydrodiols (BJAC88). Ring oxidation reaction for thiophenes seem to be directed away from the K-region.

No information is available on the phase II metabolic processing of thiophene metabolites. The sulphoxide and sulphone products may be sufficiently water soluble to be excreted without further processing. Given the requirement of a nucleophylic center for sulpho and glucuronide transferases, it is unlikely that the sulpho-oxidized metabolites would serves as effective conjugate receptors. Ring oxidation products can be expected to be subject to phase II processing in the same way as their classical PAH analogues.

Table D.1.4-1: Metabolic products of Nitrogen and Sulphur containing heterocyclic PAH.

Substrate	Product	Enzyme	Reference
Nitro Heterocyclic PAH	Epoxides	Cytochrome P-450s	BLEH88, JWOO86, JWOO89, JDUK88
Nitro Heterocyclic PAH	Dihydrodiols	Epoxide Hydrolase	BLEH88, JWOO86, JWOO89, JDUK88
Nitro Heterocyclic PAH	Dihydrodiol-Epoxides	Cytochrome P-450s	BLEH88, JWOO86, JWOO89, JDUK88
Nitro Heterocyclic PAH	Phenols	Cytochrome P-450s	BLEH88, JWOO86, JWOO89, JDUK88
Nitro Heterocyclic PAH	*bis*-Dihydrodiols	Cytochrome P-450s	BLEH88
Sulpho Heterocyclic PAH	Sulphoxide	Cytochrome P-450b	JBAC88, JBER86
Sulpho Heterocyclic PAH	Sulphone	Cytochrome P-450s	JBAC88, JBER86
Sulpho Heterocyclic PAH	Dihydrodiols	Cytochrome P-450s	JBAC88
Sulpho Heterocyclic PAH	Phenols	Cytochrome P-450s	JBAC88

D.2 The Enzymes of PAH Metabolism

D.2.1 Phase I Metabolic Enzymes

D.2.1.1 Cytochrome P-450

The cytochrome P-450 family of enzymes are central to the oxidative metabolism of all classical and most substituted PAH (BCOO83, BNEM81). While studies have shown that cytochrome P-450 metabolism can occur at most, if not all, of the aromatic double bonds in a PAH molecule, reactions occur primarily at the periphery (BEST78). The major products of cytochrome P-450 medicated PAH metabolism are simple epoxides. These products can either undergo spontaneous rearrangement to form phenolic products or react enzymatically with water to form dihydrodiols or with glutathione to form conjugated products (BEST78). The following sections provide a summary of cytochrome P-450 function and distribution.

D.2.1.1.1 Cytochrome P-450: Cellular and Tissue Distribution

Cytochrome P-450 is an integral membrane protein located in the endoplasmic reticulum (E.R.) (JGRO86, BEST78). It has also been found in preparations of nuclear membranes (JGRO86). Studies of highly purified nuclear cytochrome P-450 suggests that it is the same enzyme as that found in the E.R. (JGRO86).

Cytochrome P-450 has been found in may tissues including liver, lung, kidney, testis, skin and gut (JFLE90, JGRO86, BYAN78, JWOO89, JDUK88). In general hepatic tissue has the highest levels of cytochrome P-450, which is in keeping with the liver's function as the primary site of xenobiotic detoxification (JWOO89). Cytochrome P-450 levels in extrahepatic tissue are generally about 10 percent of those found in hepatic tissue. A more detailed discussion of cytochrome P-450 tissue profiles can be found in section D.3.2 of this document.

D.2.1.1.2 Cytochrome P-450: Enzyme Multiplicity

Early studies of the cytochrome P-450 system demonstrated that the enzyme had a very broad range of substrate specificities (BCOO83, JCHR87). It is responsible for xenobiotic detoxification reactions in additions to the oxidation of numerous endogenous compounds including; fatty acids, steroids, vitamins, bile acids leukotrienes and prostaglandins (JRYA90). Subsequent identification of numerous cytochrome P-450 isozymes has helped to explain how this multiplicity is achieved (BCOO83, JGUE82, JGUE81, JVLA82, JWAN83, JIMA86, JIMA87, JHAL88, JWIL87, JPAR83, JCHR87).Since the identification of multiple forms of cytochrome P-450, greater than twenty hepatic isozymes have been identified in the rat. However, the total number of isozymes that exist in any given tissue remains unknown (JRYA90).

Labelling cytochrome P-450 isozymes has not yet been standardized. A multitude of cytochrome P-450 nomenclature formats exist in current literature. Frequently, the names used reflect the species being studied and the types of induction treatment used. This makes it difficult to compare results between studies. Nebert and co-workers have attempted to clarify the problem by developing a nomenclature based on gene families (JNEB87). While this approach is a major step forward in correlating inter-species differences and similarities in cytochrome P-450 structure and function, it has not yet gained wide acceptance. Therefore using this system to compare data between reports is, as yet, impractical. Consequently, a different system must be employed.

This document will use the identification scheme employed by Ryan and co-workers because it is the simplest to follow (JRYA90). While this system does not identify all cytochrome P-450 isozymes, it does identify twelve of the major cytochrome P-450 isozymes found in hepatic and extrahepatic tissue in the rat and other species. The twelve isozymes are distinguished by the use of lower case letters immediately following P-450 as; Cytochromes P-450a, P-450b, P-450c, P-450d, P-450e, P-450f, P-450g, P-450h, P-450i, P-450j, P-450k and P-450p. Although this system is not as descriptive as the gene-family scheme, it is in common usage in the literature. In addition, it is frequently used as a benchmark by other groups. As a result, this system is a useful means of comparing results between reports.

Although many cytochrome P-450 isozymes have been identified, only a small number (cytochromes P-450a-e) are responsible for the metabolism of most xenobiotics (JPAR83, JRYA79, JCHI86). These isozymes are present in limited amounts in tissue from control animals but are inducible by some xenobiotic insults (JPAR83, JRYA79, JCHI86). Phenobarbitol (PB) and 3-methylcholanthrene (CAS #

56-49-5)(3-MC) are two inducers commonly used to examine cytochrome P-450a-e associated enzyme activities (JPAR83). A list of cytochrome P-450 isozymes responsible for xenobiotic metabolism, their responses to PB and 3-MC induction and their metabolic activity towards PAH is provided in table D.2.1.1.

Table D.2.1.1: Primary Xenobiotic Metabolizing Cytochrome P-450 Isozymes.

Isozyme	Inducer	Activity Toward PAH	Ref
Cytochrome P-450a	PB, 3-MC	None	JRYA79, JCHI86, JRYA90
Cytochrome P-450b	PB	Minor hydroxylation of methyl groups on some PAH	JRYA79, JCHI86, JCHR87
Cytochrome P-450c	3-MC	Primary PAH metabolizing isozyme	JRYA79, JCHI86, JRYA90
Cytochrome P-450d	3-MC	None	JCHI86
Cytochrome P-450e	PB	None	JCHI86

Cytochrome P-450a is found in minor amounts in hepatic tissue of animals treated with both PB and 3-MC. It does not appear to have any specificity for PAH compounds (JRYA79, JCHI86, JRYA90). Cytochrome P-450b is the major PB inducible form (JPAR83, JRYA79). Cytochrome P-450e appears to be co-induced with cytochrome P-450b. Neither isozyme shows any appreciable activity towards PAH (JPAR83, JRYA79). However, at least one report does suggest that cytochrome P-450b does exhibit a minor hydroxylation at the 12 methyl group of 7,12-dimethylbenzanthracene (CAS # 57-97-6) (DMBA) (JCHR87). Cytochrome P-450c is the major form found in animals treated with 3-MC (JPAR83, JRYA79, JRYA90). In rat hepatic tissue, treatment with 3-MC can lead to a 20 to 30 fold increase in cytochrome P-450c associated enzyme activities (JRYA79). Cytochrome P-450c shows a very high PAH hydroxylation activity (JRYA79, JCHI86). Cytochrome P-450d is a minor isozyme induced by 3-MC. Levels of this isozyme do not appear to be affected by treatment with PB (JCHI86). Despite being marginally induced by 3-MC, cytochrome P-450d does not show any appreciable activity towards PAH (JCHI86). Because cytochrome P-450c is the isozyme which is primarily responsible for PAH metabolism, this report will focus on this isozyme and its associated enzyme activities.

D.2.1.1.3 Cytochrome P-450: Specificity and Mechanism of Action

Oxidation by cytochrome P-450 requires reducing equivalents, molecular oxygen and a suitable substrate. A detailed discussion of the various stages in the process has been presented by Estabrook *et al.* (BEST78). The following is intended as a brief summary of the work presented by Estabrook *et al.* . Cytochrome P-450 functions in cyclical manner passing reducing equivalents from NADPH to molecular oxygen and a substrate. The stages involved in this process are outlined below.

Stage I: The low spin form cytochrome P-450 (Fe^{+3}) reacts reversibly with a substrate (R) to produce a high spin complex (Fe^{+3}-R).

Stage II: The (Fe^{+3}-R) high spin complex is reduced by an electron from NADPH to produce a ferrous cytochrome P-450 complex (Fe^{+2}-R).

Stage III: The (Fe^{+2}-R) complex reacts with molecular oxygen to produce a ternary complex (Fe^{+2}-R-O_2).

Stage IV: The (Fe^{+2}-R-O_2) complex receives an additional electron from NADPH to produce (Fe^{+2}-R-$O_2^{=}$); where the oxygen is in a two electron reduced state called a peroxide anion.

Stage V: Little is known about the final electron rearrangement. Protonation of the peroxide anion (possibly from a hydrogen from the substrate) results in the formation of a hydrogen peroxide and a regeneration of the high spin ferric hemoprotien-substrate complex. The final rearrangement results in the production of the oxidized substrate and water; where one atom of molecular oxygen is incorporated into the substrate to produce an epoxide and a hydrogen is released from the substrate. The hydrogen presumably is combined with the hydroxyl from the previously formed peroxide to produce a molecule of water.

The existence of multiple cytochrome P-450 isozymes explains, in part, the broad range of substrate specificities seen within the cytochrome P-450 family. However, each isozyme also has a broad range of substrate specificities. The shape, hydrophobicity and chirality of the active site play a large role in determining the types of substrates which are suitable (BYAN78). Substrate specificity and the factors which govern it have been reviewed by Yang *et al.* (BYAN78).

D.2.1.1.4 Cytochrome P-450: Regulation

The cytochrome P-450 isozymes responsible for the metabolism of most xenobiotics (cytochromes P-450a - P-450e) are present only in limited amounts in control animals (JPAR83, JRYA79, JCHI86, JIOA90). There are a number of factors which affect the levels of cytochrome P-450 including; age, sex and nutritional status (JIOA90). In all animal species including humans, cytochrome P-450 activities are low at birth but increase rapidly to adult levels. These levels decline in geriatric animals (JIOA90). Development differs between cytochrome P-450 isozymes. In the rat, cytochrome P-450c reaches maximum levels approximately two weeks postpartum and then decrease with age. Cytochrome P-450b levels are low at birth and increase with age (JIOA90). The increases in cytochrome P-450c levels in the neonatal rat matches the increase in Ah receptor levels which reach maximal levels one to three weeks postpartum and then decrease with age (JIOA90).

Studies using substrates suitable for cytochrome P-450b have shown that in male rats, cytochrome P-450b activities are greater than those found in the female (JIOA90). Cytochrome P-450c has been shown to be higher in the female than in the male (JIOA90). These results suggest that cytochrome P-450b and cytochrome P-450c have differing sexual distributions (JIOA90). Ioannides and Parkes (JIOA90) suggest that noted differences in the rates of xenobiotic metabolism between the sexes may be a reflection of differences in the distribution of cytochrome P-450 isozymes between the sexes. Nutrition and diet may also have an effect on the levels of cytochrome P-450 isozymes. These effects may be a result of chemicals inherent in the diet, food additives or compounds generated in the cooking process which are able to induce cytochrome P-450 activity (JIOA90).

The levels of the various cytochrome P-450 isozymes; in addition to being affected by the factors discussed above, may also be increased in response to xenobiotic insult. Not all inducers affect all cytochrome P-450 isozymes. Most are able to induce only specific isozymes. Phenobarbitol (PB) is able to induce both cytochrome P-450b and cytochrome P-450e. Studies have shown that these two enzymes are under coordinate control; that is, induction of cytochrome P-450b always results in a concomitant induction of cytochrome P-450e (JIOA90). In addition, the level of cytochrome P-450b induction is usually two fold greater than that seen for cytochrome P-450e (JIOA90). Neither cytochrome P-450b nor cytochrome P-450e are involved in PAH metabolism and neither appears to respond to PAH induction. Cytochrome P-450c and cytochrome P-450d appear to co-induced by a number of xenobiotics including PAH (JIOA90). However, unlike cytochrome P-450b and cytochrome P-450e, cytochrome P-450c and cytochrome P-450d are not under coordinate control (JIOA90). Studies have demonstrated that treatment with various inducers results in differing cytochrome P-450c/P-450d induction ratios (JIOA90). If these two isozymes were under coordinate control, the cytochrome P-450c/P-450d ratio would remain constant.

Cytochrome P-450c is the isozyme responsible for the activation of numerous xenobiotics including PAH. As a result, the mechanism of induction has receives a great deal of scientific attention. Evidence shows that the induction of cytochrome P-450c is regulated through the Ah receptor mechanism (JIOA90). The regulation and induction of cytochrome P-450c has been extensively reviewed by Ioannides and Parkes (JIOA90). The discussion here is intended only as a brief summary. For a more detailed review of cytochrome P-450c regulation, the reader is referred to the work of Ioannides and Parkes (JIOA90).

Numerous reports have demonstrated that the induction of cytochrome P-450c stimulates *de novo* protein synthesis (JIOA90). This protein synthesis is achieved through increases in the rate of transcription (JIOA90). Compounds which induce cytochrome P-450c bind to the Ah receptor in the cytosol. The resultant complex is translocated to the nucleus where it interacts with a structural gene thereby increasing transcription of the cytochrome P-450c genes (JIOA90). There is a good correlation between the amount of complex translocated to the nucleus and the amount of cytochrome P-450c produced (JIOA90). The Ah receptor protein has been demonstrated in numerous species including rat, mouse and human (JIOA90). Studies have shown that the receptor proteins are similar in these species, but they are thought to have differing affinities for certain ligands (JIOA90). The exact nature of the Ah receptor protein remains unknown, but it is thought that it shares many of the structural and physicochemical properties with steroid receptors (JIOA90).

D.2.1.2 Epoxide Hydrolase

Epoxide hydrolase[1] (E.H.) EC 3.3.2.3) catalyzes the hydration of a wide range of epoxides on aromatic and olefinic double bonds to their corresponding *trans*-dihydrodiols (JOES81, JWAL78, JWAE88). Therefore it plays a critical role in PAH metabolism by generating dihydrodiols which can either be excreted or serve as precursors of diol-epoxides (JWAL78, JOES77, JOES83, JWAE88). Primary epoxides of PAH are relatively lipophilic and have a tendency to remain in the endoplasmic reticulum after formation (JBEN76). Epoxide hydrolase is located in the same cellular compartment as the cytochrome P-450 system which generates the epoxides (JOES77, JOES83). Therefore, the activity of E.H. may serve to control the levels of epoxides which accumulate, by converting them to dihydrodiols, thereby enhancing their removal and reducing their potential for interacting with DNA (JOES77, JBEN76). This section provides an overview of epoxide hydrolase cellular location, enzyme multiplicity, specificity and regulation.

D.2.1.2.1 Epoxide Hydrolase: Cellular and Tissue Location

Epoxide hydrolase activities have been detected in cytosolic and membrane preparations from hepatic and extrahepatic tissues in rats and mice (JOES83). Membranous EH activity has been identified in the ER as well as in the plasma, nuclear, mitochondrial and golgi membranes (JOES83).

Epoxide hydrolase is widespread in mammalian tissue (JOES77, JOES83). The organ distribution of EH between species show quantitative differences, but generally the highest levels of activity are found in the liver and testis of all species (JOES81). Kidney, lung and skin have also been reported to have reasonable levels of E.H. activity (JOES81). A detailed discussion of E.H. tissue profiles in rats, mice and other species is provided in section D.3.3.

D.2.1.2.2 Epoxide Hydrolase: Enzyme Multiplicity

Two forms of E.H. have been identified; a membrane bound form (E.H.m) and a cytosolic form (E.H.c) (JGRO865, JOES81, JOES83). The apparent molecular weights and chormatographic properties of the two forms indicate that they are distinct (JGRO865, JOES81, JOES83). In addition, there is evidence that the two forms can be distinguished immunologically in both rats and mice (JOES83). It is thought to be unlikely that the cytosolic form of E.H. plays a major role in the metabolism of PAH as it is unable to convert (+/-) 4,5-dihydro-4,5-epoxybenzo[*a*]pyrene (B[*a*]P-4,5-E) (CAS # 64437-52-1) to the corresponding (+/-) *trans*-4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene (B[*a*]P-4,5-diol) (CAS # 50700-50-9) (9). The membrane bound form of E.H. is able to carry out this conversion (JOES81). Further, a lipophilic compound such as B[*a*]P-4,5-E would be expected to remain in the lipid layer (JSCH78, JBEN76). Therefore, it is unlikely that E.H.c would have access to PAH metabolites formed by cytochrome P-450c (JGRO86). For this reason, this report will focus on the membrane bound form (E.H.m) and will use the term "*Epoxide Hydrolase*" to signify this form exclusively, throughout this report.

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1 Epoxide Hydrolase and Epoxide Hydratase are both used in the literature to identify the same enzyme activity. This report has adopted Epoxide Hydrolase

Epoxide hydrolase is a constitutive membrane protein which is found in numerous cellular membranes (see section D.1.2.1). Within this group there appears to be a limited level of diversity. Mitochondrial E.H. has been shown to resemble cytosolic E.H. (JOES83). Epoxide hydrolase found in plasma, nuclear and Golgi apparatus membranes appear to be the same as those located in the ER. Studies which have examined EH in multiple species suggest that human and rat E.H. may arise from the same gene product and that differentiation is achieved through post-translational modification (JDUB82).

D.2.1.2.3 Epoxide Hydrolase: Specificity and Mechanism of Action

Although E.H. has a broad substrate specificity, it does place rigid structural requirements on a substrate (JOES81). For example, *cis*-1,2-disubstituted epoxides are good substrates while *trans*-1,2-disubstituted, tri and tetra substituted epoxides are poor substrates (JOES81). Primary epoxides of PAH which are structurally similar to *cis*-1,2-disubstituted epoxides are good substrates for E.H. (JOES81). This includes a number of K-region and non K-region epoxides (see table D.2.2.1). Indeed, the conversion of B[*a*]P-4,5-E to its corresponding dihydrodiol has been a standard measure of E.H. activity (See Section D.3.3). The affinity of E.H. for the K-region 4,5-epoxide and bay region 7,8 and 9,10-epoxides of B[*a*]P is approximately the same. However, the affinity for the 11,12-dihydro-11,12-epoxybenzo[*a*]pyrene is lower than for the others (JOES81). Epoxide hydrolase does not significantly increase the rate of conversion of diol-epoxides to the corresponding tetrols (9). Indirect evidence suggests that E.H. has little activity towards bay-region diol-epoxides (JOES81, JOES83).

Products from E.H. reactions are often highly stereo-selective (JOES81). Rat microsomal preparations convert (+/-)-7,8-dihydro-7,8-epoxybenzo[*a*]pyrene (CAS#) exclusively to the (-) enantiomer (absolute configuration 7R,8R) (JGRO86). Conversion of the K-region B[*a*]P-4,5-e produces both (+) and (-) dihydrodiols in approximately the same amounts (JGRO86). Further investigation has shown that B[*a*]P-7,8-E is hydrated by E.H. specifically at C8 while the B[*a*]P-4,5-E is hydrated equally well at both C4 and C5 (JGRO86).

Table D.2.2.1: PAH Epoxide Substrates for Epoxide Hydrolase (JOES83)

Compound	Region
Benz[a]anthracene, 4,5-dihydro-4,5-epoxy-, (+/-)	K
Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-, (+/-)	K
Benz[a]anthracene, 4,5-dihydro-4,5-epoxy-7-methyl-, (+/-)	K
Dibenz[a,h]anthracene, 4,5-dihydro-4,5-epoxy-, (+/-)	K
Cholanthrene, 11,12-dihydro-11,2-epoxy-3-methyl-,(+/-)	K
Phenanthrene, 9,1-dihydro-9,10-epoxy-, (+/-)	K
Benzo[a]pyrene, 7,8-dihydro-7,8-epoxy-, (+/-)	Non K
Benzo[a]pyrene, 9,10-dihydro-9,10epoxy-, (+/-)	Bay
Benzo[a]pyrene, 11,12-dihydro-1,12-epoxy-, (+/-)	Bay

D.2.1.2.4 Epoxide Hydrolase: Regulation

Epoxide hydrolase appears to be under separate genetic control from the cytochrome P-450 system (JOES81). Epoxide hydrolase levels are inducible, but not to the same extent as cytochrome P-450 (JGRO82, BSIM81, JPAR83). Both PB and 3-MC are able to induce E.H. levels, but the levels of induction are significantly lower than that seen with cytochrome P-450 (JPAR83).

Hepatic E.H. is essentially uninducible in neonate rats. Enzyme levels increase as the animals mature (JOES81). In adults, enzyme levels are usually two to three fold higher in the male than in the female (JOES81). Both of these observations may be explained by the role of E.H. in the production of hydroxylated steroids which are primarily male sex hormones (JOES81).

D.2.2 Phase II Metabolic Enzymes

Many of the PAH metabolites produces as a result of Phase I enzyme action are not sufficiently water soluble to be excreted without further modification (JGRO86, JGEL80, JDEP78). Phase II conjugation reactions which produce glutathione, glucuronyl and sulpho-derivatives generally increase water solubility sufficiently to facilitate excretion (JGRO86, JGEL80). The three groups of enzymes; glutathione-S-transferases (GST), UDP-glucuronyl transferases (UDP-GT) and sulphotransferases (ST) are discussed individually in the following sections. Detailed discussions of the tissue profiles of each group can be found in sections D.3.4 through D.3.6.

D.2.2.1 Glutathione-S-Transferase

The name Glutathione-S-Transferase (GST) (EC 2.5.1.1.8) encompasses a number of enzymes all of which catalyze the addition of a nucleophilic glutathione to a wide range of electrophilic receptors (JKET86). In PAH metabolism, GSTs are able to conjugate a number of primary anD secondary epoxides thereby preventing either the further activation of these compounds of their interaction with biological macromolecules including DNA (JMOR87, JKET88, JVOS90). This removal of potentially reactive molecules may have an important role in determining the ultimate activity of a PAH molecule (JMOR87).

D.2.2.1.1 Glutathione-S-Transferase: Cellular and Tissue Location

Glutathione-S-transferases have been identified in both cytosol and in ER and outer mitochondrial membranes (JGRO86, BSIM81, JMOR87). Cytosolic GST can account for up to ten percent of cytosolic protein (JGRO86, BSIM81). In ER microsomal preparations, GST can account for up to five percent of the membrane protein (JMOR87). It has been suggested that membrane bound GST may play a greater role in the conjugation of PAH metabolites than the cytosolic form, as the membrane bound form will have greater access to the lipophilic PAH metabolites than would cytosolic GST (JMOR87). Although this appears to be a rational assumption, there is, as yet, no data to confirm its validity. Apart from possibly having greater access to PAH metabolites, the membrane bound GST does not appear to differ significantly form the cytosolic form of the enzyme (JMOR87).

Glutathione-S-transferases are widely distributed in many tissues throughout the animal kingdom (JGRO86, BSIM81). They can be found in liver, intestinal mucosa, lung, adrenal, testis, thymus and spleen in rats, mice, rabbits, humans and other vertebrates (JGRO86, BSIM81, JMOR87, JKET88). In rats, hepatic GST levels have been reported to be several hundred times greater than those in extra-hepatic tissue (JMOR87). The tissue distribution of GST will be discussed in greater detail in section D.3.4.

D.2.2.1.2 Glutathione-S-Transferase: Enzyme Multiplicity

Much of the work on GST has been conducted in rat hepatic tissue. Consequently, it is the best understood system and one which frequently serves as a standard against which other GST enzymes are compared (JKET88). A detailed discussion of all aspects of GST multiplicity in rats and other species is well beyond the scope of this report. Multiplicity has been well reviewed elsewhere (JKET86, JKET88,JVOS90,JMOR87). A brief summary of rat hepatic GST multiplicity is provided below.

Rat hepatic GST has been shown to be composed of two subunits. These subunits do not have to be the same (JKET88). To date, ten individual GST subunits have been identified in rat hepatic tissue (JKET88). Each subunit has also been found in every extra-hepatic tissue examined thus far (JVOS90). However, differences in subunit profiles appear to exist between tissues (JVOS90). It is unlikely that differences in subunit profiles between tissues will contribute to differences in tissue sensitivity to PAH as all subunits appear to be able to conjugate B[a]P primary epoxides (JKET88).

D.2.2.1.3 Glutathione-S-Transferase: Specificity and Mechanism of Action

Glutathione-S-transferase are responsible for transferring a glutathione moiety to an acceptable electrophilic receptor (JGRO86, BSIM81, JKET88, JVOS90). Thus, GST enzymes have an absolute requirement for glutathione (JGRO86, JKET88). They do however, have a wide and overlapping secondary substrate specificity (JGRO86, BSIM81, JKET88). Simple (primary) epoxides and diol-epoxides are both capable of serving as secondary substrates for GST (JGRO86). The GST enzymes act directly upon the epoxide ring, opening it through an electrophilic substitution of the oxygen with the sulphur atom of glutathione (JGRO86, JKET88). Ring opening generates a glutathione-S-PAH conjugate with a hydroxyl group on the carbon at the site of ring opening. The conjugated product is subject to further metabolic processing; glutamic acid and glycine residues are removed from the glutathione leaving a cysteine residue which is subsequently acetylated to form a mercapturic acid which is excreted in the urine (JNEM81).

Epoxides of varying complexity and differing molecular location will differ in their ability to act as secondary substrates (JGRO86, JNEM81). Epoxides located in the K-region of a PAH are generally good substrates for GST enzymes. Non-K-region epoxides are poorer substrates (JGRO86, JNEM81). Phenols, dihydrodiols and quinones are very poor GST substrates because these metabolites do not possess the requisite electrophilic centers on the molecule (JNEM81). A partial list of GST substrates is shown in table D.2.2.1.

Table D.2.2.1: A Partial List of PAH Metabolites Suitable For GST

Compound	Region	Substrate	Ref
Phenanthrene, 9,10-dihydro-9,1-epoxy-, (+/-)	K	Good	JGRO86, JNEM81
Benz[a]anthracene, 5,6-dihydro-5,6-epoxy-, (+/-)	K	Good	JGRO86, JNEM81
Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-, (+/-)	K	Good	JNEM81
Cholanthrene, 11,12-dihydro-11,12-epoxy-3-methyl-, (+/-)	K	Moderate	JNEM81
Dibenz[a,h]antracene, 5,6-dihydro-5,6-epoxy-, (+/-)	K	Moderate	JNEM81
Benzo[a]pyrene, 7,8-dihydro-7,8-epoxy-, (+/-)	Non-K	Moderate	JNEM81
Benzo[a]pyrene, 9,10-dihydro-9,10-epoxy-, (+/-)	Bay	Moderate	JNEM81
Benzo[a]pyrene, 11,12-dihydro-11,12-epoxy-, (+/-)	Bay	Moderate	JNEM81

D.2.2.1.4 Glutathione-S-Transferase: Regulation

Glutathione-S-transferase subunits have been found in many fetal tissues although early work suggested that subunits showed varying rates of fetal maturation (JGRO86). Glutathione-S-transferase have been shown to be inducible by a number of xenobiotic insults including PB and 3-MC (JKET88). There does not appear to be any data regarding differences in subunit induction in response to either PB or 3-MC treatment.

D.2.2.2 UDP-Glucuronyl Transferases

Glucuronide formation increases the water solubility of many endogenous and xenobiotic substances, making them easier to excrete than the parent compounds (JTEP89, JBOC91). The conjugation of PAH metabolites by UDP-glucuronyl transferases (UDP-GT) (EC 2.4.1.17) is an important pathway in the elimination of PAH (JGRO86). Removal of PAH metabolites by this pathway prevents their further oxidation to more reactive products (JBOC91). Glucuronic acid conjugates of PAH are generally regarded as detoxification products as further activation is no usually possible (JGRO86). However, the enzymatic hydrolysis of 3-hydroxy-B[a]P-glucuronide by gut microflora yields a cytotoxic product capable of binding to DNA if reabsorbed across the gut wall (JGRO86).

D.2.2.2.1 UDP-Glucuronyl Transferases: Cellular and Tissue Location

UDP-Glucuronyl transferases are integral membrane proteins located in the E.R. (JGRO86, BCOO83, JNEM81, JTEP89). Evidence suggests that UDP-GT are in relatively close proximity to cytochrome P-450 (JTEP89). This would allow UDP-GT easier access to the primary and secondary PAH metabolites of the cytochrome P-450/epoxide hydrolase system (JNEM81). Little is known about the proximity of UDP-GT to E.H.. However, because E.H. is thought to be in reasonably close proximity to cytochrome P-450 (JOES83), it is reasonable to assume that E.H. and UDP-GT are also closely associated.

UDP-Glucuronyl transferases have been found in many tissues including; liver, lung, kidney, stomach, intestinal mucosa and skin (JGRO86, BCOO83, JNEM81). They do not appear to be in either brain or spleen (BCOO83, JNEM81). A more detailed discussion of the available UDP-GT tissue profile data is presented in Section D.3.5

D.2.2.2.2 UDP-Glucuronyl Transferases: Enzyme Multiplicity

A detailed discussion of UDP-GT multiplicity is beyond the scope of this report. The multiplicity has been reviewed elsewhere (JTEP89,JBOC91). The following is intended only as a brief summary. Each species appears to have its own pattern of UDP-GT isoforms (JTEP89, JBOC91). Purification of the isoforms to apparent homogeneity, sequencing and cloning have determined that UDP-GT isoforms arise from a supergene family similar to that seen with cytochrome P-450 (JTEP89, JBOC91). Each isoform appears to be relatively specific for an endogenous substrate, but all forms appear to have broad and overlapping specificities for exogenous substrates (JTEP89,JBOC91).

Data regarding the tissue distribution of the various UDP-GT isoforms is limited (JBOC91). There is some limited evidence which suggests that data of this nature may be essential for a more complete understanding of the differences in tissue and species sensitivity to PAH. For example; in the rat, UDP-GT activity against phenols seems to be distributed throughout most body organs and tissues (JBOC91). Other UDP-GT activities would appear to be restricted to the liver and intestinal mucosa (JBOC91). Therefore differences in the ability to remove specific PAH metabolites with certain UDP-GT isoforms may result in altered sensitivity. Not enough data is available to examine this further.

D.2.2.2.3 UDP-Glucuronyl Transferases: Specificity and Mechanism of Action

UDP-Glucuronyl transferases incorporate a glucuronic acid moiety into a co-substrate molecule thereby increasing the water solubility if the co-substrate (JNEM81, JBOC91). Thus UDP-GT enzymes have an absolute requirement for activated glucuronic acid. Co-substrates for the conjugation reaction are generally hydroxylated compounds, although amino, hydroxy-amino and sulfhydryl compounds can act as conjugation sites (JNEM81). The large number of primary and secondary metabolites produced by Phase I reactions provides a wide spectrum of co-substrates for UDP-GT. Consequently, a large number of conjugation products are formed (JNEM81). Conjugates formed in this manner are excreted in both bile and urine (BCOO83).

UDP-Glucuronyl transferase are able to conjugate quinone, dihydrodiol and phenolic derivative of PAH (JNEM81). Examination of UDP-GT activity towards B[a]P metabolites has demonstrated that both phenolic and quinone metabolites are preferentially removed. However, UDP-GT activity towards B[a]P dihydrodiols is lower (JGRO86). The rate of (±) trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (CAS# 61443-57-0) (B[a]P-7,8-diol) conjugation is twenty percent of that noted for 9-hydroxybenzo[a]pyrene (CAS# 17573-21-6) (B[a]P-9-OH) (JGRO86). The activity of UDP-GT towards dihydrodiols increases when UDP-GT levels are very high (JNEM81). Substituted PAH such as nitro and amino PAH contain sufficiently reactive centers that conjugation reactions can take place without prior Phase I activation (JNEM81, JBOC91, JTEP89, JBUR91). This, however, does not preclude their passing through Phase I prior to conjugation (JNEM81).

D.2.2.2.4 UDP-Glucuronyl Transferases: Regulation

Experimental work has indicated that rat liver UDP-GT enzymes can be differentially induced by both PB and 3-MC (JBOC91,JTEP89). Evidence suggests that the induction of cytochrome P-450 and UDP-GT may be genetically linked (JGRO86). Studies which have examined UDP-GT induction have shown that it may be under the control of the Ah receptor mechanism (JBOC91). Therefore induction of cytochrome P-450 would appear to be controlled by the same mechanism as that which controls UDP-GT. This would mean that an anticipated increase in the production of Phase I reactive metabolites would be met with an increase Phase II capacity to conjugate and remove Phase I products.

D.2.2.3 Sulphotransferases

Sulphotransferases (ST) are responsible for the catalytic addition of a sulphate group to many endogenous compounds (JJAK90). Sulpho-conjugation of PAH metabolites is an important removal mechanism for phenolic and dihydrodiol derivatives (JGRO86, JNEM81). The sulphate esters of classical PAH are not reactive products and can therefore be considered as true detoxification products (JNEM81). However, sulpho-conjugation has been implicated in the activation of amino substituted PAH (JGRO86).

D.2.2.3.1 Sulphotransferases: Cellular and Tissue Location

Sulphotransferases are localized in the cytosol of cells throughout the body (JGRO86, JNEM81). They play a major role in the formation of mucopolysaccarides and chondroitin sulphate, both of which are important constituents of connective tissue (JNEM81). Sulphotransferases also play a role in the sulphation of xenobiotics (JNEM81). A review of the available ST tissue profile data can be found in Section D.3.6. Despite the apparent importance of this removal mechanism, little tissue profile data is available.

D.2.2.3.2 Sulphotransferases: Enzyme Multiplicity

Many ST appear to exist (JJAK90). Two isozymes, active against phenolic compounds have been identified (JJAK90). Another three have been found to have activity against primary alcohols and hydroxylated steroids. Still other isozymes have been identified which exhibit activities against bile acids, phenolic steroids and amines (JJAK90). It is unclear at this time how many ST isozymes exist, or what range of substrate specificity each possesses (JJAK90).

D.2.2.3.3 Sulphotransferases: Specificity and Mechanism of Action

As noted above, the numerous ST isozymes appear to have very broad ranges of substrate specificity (JJAK90). Regardless of the co-substrate, all ST appear to carry out the same basic reaction; the addition of a sulphate group to a suitable nucleophilic center on a co-substrate (JJAK90,10). Hydroxyl or amino groups are the most common recipients of sulphate groups (JNEM81).

Classical PAH do not possess the required nucleophilic centers and must pass through Phase I metabolism before they undergo ST conjugation (JNEM81). Quinone and phenolic derivatives of PAH can act as substrates for ST enzymes. Quinones must first be partially reduced to semi-quinones or fully reduced to dihydroxy derivatives before they are acceptable as ST co-substrates (JGRO86, JNEM81). Sulphate conjugates of dihydrodiols appear as sulpho-conjugates of catechols, suggesting that they arise through dehydrogenation of a dihydrodiol to a catechol (JGRO86). Therefore, ST activity towards dihydrodiols is dependent upon the ease with which the dihydrodiol can be converted to the corresponding catechol (JGRO86, JNEM81). For example; the rate of formation of sulpho-conjugates of B[a]P-4,5-diol is three and eight times faster that the rates of conjugation of B[a]P-9,10-diol and B[a]P-7,8-diol respectively (JNEM81). The polarity of the sulpho-conjugate is greater than that of the parent compound, but not as great as that of the glucuronic acid conjugates (JNEM81).

D.2.2.3.4 Sulphotransferases: Regulation

Sulphotransferases do not appear to be inducible by xenobiotic insult (JNEM81). However, there is evidence which suggests that ST many be under hormonal control (JNEM81). Differences in ST levels between male and female rats has been reported in the literature (JNEM81, JMUL86). Administration of testosterone to female rats produced a one hundred percent increase in ST activity. The same treatment in male rats had no apparent effect on ST levels (JNEM81). Therefore cAre must be taken when comparing tissue profile data between sexes.

D.3 Tissue Profiles for Phase I and Phase II Enzymes

The main objective of this section is to provide answers to some of the questions outlined in Section D.1. These are outlined in table D.3.1. The remaining questions outlined in Section D.1 will be addressed in other sections of this document.

Table D.3.1: Questions to be addressed in Section D.3

1) *What differences in Phase I and Phase II enzyme levels exist between tissues within a single species?*
2) *Do differences in isozyme profiles exist between tissues within a single species?*
3) *What effects do observed differences in enzyme and/or isozyme profiles have on PAH metabolism in different tissues?*
4) *Can differences in tissue profiles help to explain observed differences in tissue, strain and species sensitivity to PAH?*

The previous sections of the document identified the principle enzymes of PAH metabolism. It also provided some necessary background information on cellular and tissue locations as well as enzyme multiplicity, substrate specificity, mechanism of action and regulation. Each of these factors needs to be considered if a complete understanding of the relationship between tissue enzymes and PAH activation is to be achieved. Although Section D.2 provides a summary of cellular location for each of the Phase I and Phase II enzymes, it provided only a cursory summary of tissue distribution. This section will examine, in detail the available tissue distribution data for each of the enzyme groups identified in Section D.2.

A quick examination of the available literature suggests that ample data should be available to answer the questions outlined in table D.3.1. Closer examination reveals that much of the data cannot be used for one of two reasons, either; i) data were reported in a single tissue or; ii) the studies used non-standard experimental protocols or reported non-standard enzyme activities. The data selection criteria used in assembling the data for analysis are discussed in Section D.3.1. Strict adherence to the selection criteria is essential to provide data sets which could be used to meet the objectives of this section. Unfortunately, few published reports were found to have adequate data. Therefore, few firm conclusions can be drawn.

D.3.1 Data Selection Criteria

A comparison of data between reports requires that only data which conforms to a determined standard be selected. The following discussion outlines the selection criteria used in this report. Where more than one protocol could be chosen, this report attempts to provide the reasoning behind the selection of a given protocol.

Data selection was a two stage process. In the first step studies which reported enzyme activities in more than one tissue were selected. Reports identified in the first stage were examined and only those which reported data which conformed to the selected protocols were chosen. This group was used in the data analysis which is discussed in subsequent sections.

D.3.1.1 Selection Criteria: Tissue Preparation

Monitoring enzyme activity in *in vivo* systems is generally achieved by monitoring tissue enzyme activity in postmortem tissue preparations. Only studies which used the same tissue preparation (tissue homogenate, 10,00 g supernatant or microsomal pellet) were compared. Occasionally, studies using different tissue preparations were compared, but only if the data for the standard tissue preparations was contained in the same report.

D.3.1.2 Selection Criteria: Enzyme Activities

From studies which examined activities in the same tissue preparation, only those which looked at the same enzyme activity (ie used the same substrate and detection technique) were compared. Minor alterations in assay parameters such as pH and temperature can have marked effects on enzyme activities. Therefore, to make valid comparisons between studies it is essential that variations in experimental protocols be kept to an absolute minimum. The parameters used for each enzyme group are listed below.

D.3.1.2.1 Selection Criteria: Cytochrome P-450

Tissue Preparation: Tissue microsomes were the primary tissue preparation.

Enzyme Activities: Three enzyme activities were reported; aryl hydrocarbon hydroxylase (AHH), ethoxycoumarin and ethoxyresorufin-O-deethylases (ECOD and EROD). All three monitor cytochrome P-450c associated enzyme activity, the principle cytochrome P-450 involved in PAH metabolism (See section D.2)

- **AHH:** Monitors the conversion of B[a]P to hydroxylated B[a]P products. Assay protocols generally use radiometric detection.
- **ECOD:** Monitors the O-deethylation of 7-ethoxycoumarin. Assay protocols generally use fluorometric detection.
- **EROD:** Monitors the O-deethylation of 7-ethoxyresorufin. Assay protocols generally use fluorometric detection.

Total Enzyme Concentration: Total Cytochrome P-450 concentrations are measured by carbon monoxide difference spectroscopy.

Studies which reported enzyme activities other than those listed above or which reported cytochrome P-450 concentrations using a different detection technique were not considered.

D.3.1.2.2 Selection Criteria: Epoxide Hydrolase

Tissue Preparation: Tissue microsomes were the primary tissue preparation used.

Enzyme Activities: Benzo[a]pyrene-4,5-dihydro-4,5-epoxy-, (±) (B[a]P-4,5-epoxide), *cis*-stilbene oxide and styrene oxide are the most commonly used E.H. substrates. The assay monitors the conversion of the substrate epoxide to its corresponding dihydrodiol. Products are measured by radiometric detection.

Studies which use B[a]P-4,5-epoxide as a substrate were given preference as this substrate is the most relevant to PAH metabolism. As the majority of the identified papers reported using B[a]P-4,5-epoxide, giving preference to this substrate did not result in the exclusion of many reports form the final analysis.

D.3.1.2.3 Selection criteria: Glutathione-S-Transferases

Tissue Preparation: The supernatant fraction from a 105,000 x g microsomal pellet was the most common cytosolic preparation employed. Others included the supernatant from 10,000 x g crude tissue preparations (This preparation is generally referred to as S9), crude tissue homogenates and "Broken Cell" preparations. Preference was given to the 105,000 x g supernatant cytosol and S9 cytosol.

Enzyme Activities: Two artificial substrates are commonly used to measure GST activity; 1-chloro-2,4-dinitrobenzene (CDNB) and *cis*-stilbene oxide. The bulk of the identified data used CDNB as a substrate. therefore, data selection and analysis has focused on these reports as they provide the largest and most complete data set. Glutathione-s-transferase activity towards CDNB and *cis*-stilbene oxide appears to be sufficiently dissimilar that combining the two sets of data would not be feasible. The formation of product is monitored by spectrophotometric detection.

D.3.1.2.4 Selection Criteria: UDP-Glucuronyl Transferases

Tissue Preparation: Tissue microsomal preparations were used in all reports from which data was taken.

Enzyme Activities: Two substrates; 1-naphthol and 4-nitrophenol were used. Studies which used 1-naphthol were given preference as they provided the largest data set. The formation of product is monitored using fluorometric detection.

D.3.1.2.5 Selection Criteria: Sulphotransferase

Tissue Preparation: Crude tissue homogenates or S9 cytosolic preparations were used.

Enzyme Activities: 2-naphthol was used as substrate. The formation of product was monitored using spectrophotometric detection.

D.3.2 Tissue Profiles for Cytochrome P-450

The cytochrome P-450 system has been studied in a number of tissues from a variety of species. Rat, mouse and rabbit are the most commonly studied species, but data are available for numerous others including; guinea pig, hamster, dog, cat, pig, monkey and human. Cytochrome P-450s have been detected in many tissues including; liver, lung, kidney, testis, skin, gut and others (JDEP78, BCOO83, JSAB88, JLEH82, JOES77). In all species examined, cytochrome P-450 concentrations are greater in hepatic than in extra-hepatic tissues. As cytochrome P-450 is responsible for the formation of reactive PAH metabolites, differences in cytochrome P-450 concentrations between tissues may help to explain differences in tissue, strain and species sensitivities to PAH. The following sections examine the available cytochrome P-450 tissue distribution data in rats and mice. Where there is sufficient data, other species have also been examined.

D.3.2.1 Cytochrome P-450 Tissue Profiles in Rats

There are a limited number of studies which have investigated cytochrome P-450 concentrations and enzyme activities in more than one rat tissue. The studies which have adequate data and the tissues and enzyme activities they monitored are summarized in table D.3.2-1. The majority of the data summarized comes from studies which used Sprague-Dawley rats. A limited amount of tissue profile data is also available for Wistar rats. Long-Evans and Fisher 344 rats are other strains which are commonly used in cytochrome P-450 studies. However, cytochrome P-450 tissue profile data could not be located for either of these two strains. Therefore, they have not been considered. The data for Sprague-Dawley and Wistar rats will be discussed separately in order to identify inter-strain similarities and differences. Should the two strains prove sufficiently similar, the data will be combined to provide a species cytochrome P-450 tissue-profile.

Table D.3.2-1: Summary of Suitable Cytochrome P-450 Data in Multiple Tissues From Control Rats

Strain/Sex	Activity	Tissue	Ref
S.D.[1] Neonate	AHH,ECOD,[P-450t][2]	Liver, Whole Skin, Epidermis, Dermis	JBIC82
S.D. Neonate	AHH, ECOD, EROD	Liver, Epidermis	JKHA89A
S.D. Neonate	AHH, ECOD, EROD	Liver, Whole Skin	JASO85
S.D. (M) Adult	AHH	Liver, Kidney, Testis	JSUZ80
S.D. (M) Adult	AHH	Liver, Lung, Kidney	JSUN86
S.D. (M) Adult	AHH	Liver, Lung, Kidney, Pancreas	JWIL90
S.D. (M) Adult	AHH, [P-450t]	Liver, Lung, Kidney, Testis	JGOL84
S.D. (M) Adult	[P-450t]	Liver, Lung, Kidney	JCHR87
S.D. (M) Adult	[P-450t]	Liver, Lung	JKEI87
S.D. (M) Adult	[P-450t]	Liver, Prostate	JHAA83
S.D. (M) Adult	[P-450t]	Liver, Intestine	JBON85
Wistar (M) Adult	AHH, [P-450t]		JGNO84
Wistar (M) Adult	[P-450t]		JSES90

1 Sprague-Dawley
2 Total Cytochrome P-450 Concentration

D.3.2.1.1 Cytochrome P-450 Tissue Profiles in Sprague-Dawley Rats

The available data for Sprague-Dawley (S.D.) rats falls into two groups; adult males and neonates. In adults, cytochrome P-450 concentrations and AHH activity data are available for a number of extra-hepatic tissues (see table D.3.2-1). Unfortunately, there do not appear to be any reports which investigate these activities in epidermal or dermal tissue. Cytochrome P-450 concentrations, AHH, EROD and ECOD activities in epidermal and dermal tissue is available for neonate S.D. rats/ Because the hepatic data is available for both neonatal and adult animals it may be possible to compare activity between the two groups and possibly incorporate the neonatal data into the adult rat data set. In this fashion it may be possible to develop a tissue profile for adult S.D. rats which includes epidermal and dermal values.

Many reports do not distinguish between epidermal and dermal tissue but look at whole skin instead. Direct comparison between these studies and those which report only epidermal values is difficult and subject to error. Studies which report values for whole skin, epidermis and dermis have suggested that summation of the epidermal and dermal values provides a reasonable approximation of the activities found on whole skin (JBIC82). If the ratio between epidermal and whole skin cytochrome P-450 levels and activities are assumed to be constant, it then becomes possible to compare whole skin and epidermal data. It should be noted however, that there is insufficient data available to establish the validity of this assumption. Therefore, conclusions drawn using this method must be viewed with some reservations.

D.3.2.1.1.1 Cytochrome P-450 Profiles in Adult Sprague-Dawley Rats

From the total cytochrome P-450 data in table D.3.2-2 it can be seen that total cytochrome P-450 ([p-450t]) levels are highest in hepatic tissue, with a mean concentration of 781±58 pmoles per milligram of microsomal protein (pmoles/mg). Cytochrome P-450t concentrations in extra-hepatic tissues range between two and thirteen percent of the hepatic levels. The data reported by Keith *et al.* (JKEI87) has been excluded from the calculations of mean and standard error and from the subsequent normalization of the data as this report appears to be largely a duplication of the work reported by Chritow (JCHR87). Both reports share many of the same authors and it seems that a single set of data was used in both reports. Therefore, inclusion of both points would place undo weight on this data point.

Table D.3.2-2: Total Cytochrome P-450 Concentrations; Tissue Profiles in Control Adult Male Sprague-Dawley Rats

	Tissue (Cytochrome P-450 Concentration pmoles/mg[1])						
Prep.[2]	Liver	Lung	Kidney	Testis	Gut[3]	Prost[4]	Ref
Microsomes	860±90	62±12	50±70	55±15			JGOL84
Microsomes	640±160	30±10	80±15				JCHR87
Microsomes	650±160[5]	30±10[5]					JKEI87
Microsomes	735±143				84±17		JBON85
Microsomes	890					20	JHAA83
Mean±S.E.[4]	781±58	41±11	65±15	55	84	20	

1 Cytochrome P-450 concentration expressed as pmoles P-450/ mg microsomal protein.

2 Tissue preparation.

3 Intestinal tissue.

4 Prostate.

5 Data excluded from calculation of mean (see discussion).

Normalization of the data in table D.3.2-2 makes it easier to determine a tissue profile for all extrahepatic tissue listed. Normalized cytochrome P-450t concentrations are shown in table D.3.2-3. From the normalized data a partial cytochrome P-450t concentration tissue profile can be suggested as the following.

Liver (100)> Intestine (11)> Kidney (9.2)> Testis (6.4)> Lung (5.9)> Prostate(2.2)

Total cytochrome P-450 concentration data measures all cytochrome P-450 isozymes. As has already been discussed, cytochrome P-450c is the isozyme primarily responsible for PAH metabolism (see Section D.2). Therefore, tissue profiles for cytochrome P-450c are of greater interest and relevance in determining tissue PAH metabolism differences. The available AHH data for adult male S.D. rats is shown in table D.3.2-4.

Table D.3.2-3: Normalized Total Cytochrome P-450 Concentration Tissue Profiles in Control Adult Male Sprague-Dawley Rats

		Corrected Tissue Levels (pmoles [P-450t]/mg					
[P-450t][1]	N.F.[2]	Lung	Kidney	Testis	Gut[3]	Prost[4]	Ref
860	0.91	56.4	45.5	50.1			JGOL84
640	1.22	36.6	97.6				JCHR87
735	1.02				85.7		JBON85
890	0.85					17	JHAA83
Average (% Liver)[5]		46.5 (5.9)	71.6 (9.2)	50.1 (6.4)	85.7 (11.0)	17 (2.2)	

1 Total cytochrome P-450 concentration cited in reference.

2 Normalization factor: (Means [P-450t]/cited[P-450t])

3 Intestinal tissue.

4 Prostate.

5 Numbers in brackets are percent cytochrome P-450t levels found in hepatic tissue.

From the data presented in table D.3.2-4 it can be seen that there is a wide range in AHH activities in liver microsomes (115 to 607 pmole of product/minute/mg microsomal protein). As mentioned in section D.3.1, there are a number of factors which can affect the determination of enzymatic activity. This makes direct comparisons between reports difficult. However, comparisons between relative levels are possible. The data presented in table D.3.2-4 shows that AHH levels are highest in hepatic tissue. Activities in extra-hepatic tissues are less than fifteen percent of those in hepatic tissue. Extra-hepatic AHH activities are highest in lung followed by kidney, testis, intestine and spleen. Thus the tissue profile for AHH activity in adult male S.D. rats would appear to be the following.

Liver	**>>**	**Lung**	**>**	**Kidney**	**>**	**Testis**	**>**	**Intestine**	**> Spleen**
(100)		**(8.9)**		**(5.1)**		**(2.4)**		**(0.2)**	**(0.1)**

It should be noted however, that while the average value for lung AHH activity is greater than that for kidney, the ranges of relative activities for both are similar (4.1 to 13.6 % of hepatic activity for lung and 1.6 to 11.4 % of hepatic activity for kidney). Therefore, the levels of AHH activity reported in the two tissues can be considered to be equal within the range of experimental variation reported (JSUZ80, JSUN86, JGOL84).

Table D.3.2-4: Aryl Hydrocarbon Hydroxylase Tissue Profiles for Control Adult Male Sprague-Dawley Rats

	Tissue (AHH Activity = pmoles/min/mg[1])							
Prep[2]	Liver	Lung	Kidney	Testis	Gut[3]	Panc[4]	Spleen	Ref
Microsomes	115±39		1.8± (1.6)[5]	1.1±0.02 (1.0)				JSUZ80
Microsomes	220	30 (13.6)	25 (11.4)					JSUN86
Microsomes	607±80	25±5 (4.1)	12±1 (2.0)	3.7±0.1 (0.6)	0.9±0.2 (0.2)		0.4±0.1 (0.1)	JGOL84
Tiss Hom[6]	39.9					0.09		JWIL90
Avg[7]	100	8.9	5.0	0.8	0.2		0.1	

1 Enzyme Activity expressed as pmoles product formed/minute/mg microsomal protein.

2 Tissue preparation.

3 Intestinal tissue.

4 Pancreas.

5 Numbers in brackets are percent AHH activity levels found in hepatic tissue.

6 Tissue Homogenate.

7 Average extra-hepatic AHH activity levels expressed as percent of hepatic AHH activity.

The tissue profile for AHH activity suggested above differs from the tissue profile for total cytochrome P-450 concentrations in that lung and intestine appear to have changed positions in the relative ranking. Lung tissue moved from fifth position in the total cytochrome P-450 concentration profile to second in the AHH activity profile. Intestinal tissue moved from second to fifth. This suggests that while total cytochrome P-450 levels are lower in the lung than in the intestine, the percentage of the cytochrome P-450c isozyme is higher in lung tissue. The reason for this is unclear. It may help to explain, in part, the greater tumorigenicity of PAH when administered via inhalation than when administered orally (see Main Document).

Data on the levels of cytochrome P-450 and cytochrome P-450c associated enzyme activities in skin does not appear to be available for adult male S.D. rats. In order to incorporate skin data into the tissue profile presented above, data from neonatal S.D. rats will have to be considered. The next section examines the available neonatal skin cytochrome P-450 data to determine its suitability for inclusion in the adult S.D. rat tissue profile scheme.

D.3.2.1.1.2 Cytochrome P-450 Profiles in Neonatal Sprague-Dawley Rats

A limited amount of data on the levels of cytochrome P-450 and its the associated enzyme activities is available in the literature. These data are summarized in tables D.3.2-5 through D.3.2-8.

In skin, as in other extra-hepatic tissues, total cytochrome P-450 concentrations, AHH,ECOD and EROD activities are between one and ten percent of those found in hepatic tissue. Total cytochrome P-450 concentrations are higher in dermal tissue than in either epidermal tissue or whole skin (see table D.3.2-5). However, AHH and ECOD activities are lowest in dermal tissue (tables D.3.2-6 and D.3.2-7). Data on EROD activities in dermal tissue is unavailable.

Table D.3.2-5: Tissue Profiles of Total Cytochrome P-450 Concentrations in Control Neonatal Sprague-Dawley Rats

	Tissue ([P-450t] pmoles/mg protein)				
Preparation	**Liver**	**Epidermis**	**Dermis**	**Whole Skin**	**Ref**
Microsomes	230 (100)	12.4 (5.4)	21.7 (9.4)	13.7 (6.0)	JBIC82

Numbers in parenthesis are % [P-450t] in hepatic tissue

Table D.3.2-6: Tissue Profiles of AHH Activity in Control Neonatal Sprague-Dawley Rats

	Tissue (AHH Activity = pmoles/min/mg)				
Preparation	**Liver**	**Epidermis**	**Dermis**	**Whole Skin**	**Ref**
Microsomes	22±2 (100)	1.25 (5.7)			JKHA89A
Microsomes	22.6 (100)			1.67 (7.4)	JASO85
Microsomes	45.5 (100)	1.28 (2.8)	1.01 (2.2)	1.03 (2.3)	JBIC82
S9		0.62±0.05	0.42±0.03	0.51±0.02	JBIC82

Numbers in parenthesis are % AHH levels in hepatic tissue

Table D.3.2-7: Tissue Profiles of ECOD Activity in Control Neonatal Sprague-Dawley Rats

	Tissue (ECOD Activity = pmoles/min/mg)				
Preparation	**Liver**	**Epidermis**	**Dermis**	**Whole Skin**	**Ref**
Microsomes	52±4	2.2 (4.1)			JKHA89A
Microsomes	38.5			2.1 (5.4)	JASO85
Microsomes	35.2	0.93 (2.6)	0.75 (2.1)	0.83 (2.4	JBIC82
S9		0.49	0.40	0.36	JBIC82

Numbers in parenthesis are % ECOD levels in hepatic tissue

Table D.3.2-8: Tissue Profiles of EROD Activity in Neonatal Sprague-Dawley Rats

	Tissue (EROD Activity = pmoles/min/mg)				
Preparation	**Liver**	**Epidermis**	**Dermis**	**Whole Skin**	**Ref**
Microsomes	8.5	0.64 (7.5)			JKHA89A
Microsomes	10.3			0.58 (5.6)	JASO85

Numbers in parenthesis are % ECOD levels in hepatic tissue

Bickers *et al.* (JBIC82) is the only report that examines AHH and ECOD levels in epidermal and dermal tissue as well as in whole skin. Therefore it is a better comparison than studies which examine a single tissue (epidermis (JKHA89A) or Whole Skin (JASO85)). The levels reported by Bickers *et al.* show that AHH and ECOD activities are higher in epidermal tissue than either dermal tissue or whole skin (JBIC82). Comparison of the data presented by KHAN *et al.*(JKHA89A) and Asokan *et al.*(JASO85) suggests that AHH and ECOD activities are higher in whole skin than in the epidermis. However, because this comparison is made across two studies (whole skin data from Asokan *et al.* and epidermal data from Khan *et al.*) it is perhaps less accurate. The hepatic AHH activity reported by both of these studies are remarkably similar. This suggests that a comparison

between the two studies is justifiable. The differences in hepatic ECOD and EROD activities between the two studies is more pronounced (tables D.3.2-7 and D.3.2-8). Therefore, the level of confidence in a comparison between these two studies would be lower than the confidence in a study which allows a direct comparison of activities between all three tissues (epidermis, dermis and whole skin).

Although the presented data suggests that enzyme activity may be higher in epidermal tissue than in dermal tissue or whole skin, the range of reported values for all tissues suggests that the levels of enzyme activity are essentially the same in all three tissues. Data from Bicker *et al.* (JBIC82) has been used in subsequent evaluations as the level of confidence in this data is greater than that of the other studies (see above). From the data presented by Bickers *et al.* (JBIC82) it is possible to suggest a tissue profile for AHH activity in the liver and skin of neonatal S.D. rats;

Liver	**>>**	**Epidermis**	**≈**	**Whole Skin**	**≈**	**Dermis**
(100)		**(2.6)**		**(2.4)**		**(2.1)**

Incorporation of the neonatal skin data into the adult S.D. tissue profile requires that two conditions be met;

i) That hepatic AHH levels in the neonate be similar to those in the adult; or that the relationship between levels in the neonate and adult be known.

ii) That the ratio of hepatic/skin AHH activity remains constant throughout maturation.

Comparison of hepatic AHH levels between neonatal and adult S.D. rats shows that AHH levels are lower in the neonate (see tables D.3.2-4 and D.3.2-6). This is consistent with the reported observations that cytochrome P-450 levels are lower in neonatal animals than in adults (JIOA90). Although cytochrome P-450c levels are relatively high at birth, they do not reach maximal levels until two to three weeks postpartum (JIOA90). The neonatal rats used in the studies cited were between four and five days of age. Therefore it is reasonable to assume that hepatic AHH activity in the neonate would increase to the levels seen in the adult animals as the animals matured. The confidence that this condition would be met will be high as data is available which suggests that this condition holds (JIOA90).

There is insufficient data on which to evaluate the second condition as there is no data skin AHH activity in adult S.D. rats available. However, the hepatic/skin AHH activity ratio in the neonate is similar to the hepatic/extra-hepatic tissue AHH ratios found in the adult. Therefore, it would seem reasonable to assume that the hepatic/skin AHH activity ratio in the neonate will be similar to that in the adult animal. This suggests that the second condition may be met in part. The lack of adequate data means that the confidence in this assumption must be low.

Incorporating the neonatal data for Skin AHH activity into the existing AHH profile for the adult male S.D. rat provides the following suggested tissue profile;

Liver >>	**Lung >**	**Kidney >**	**Testis = Skin >**	**Intestine >**	**Spleen**
(100)	**(8.9)**	**(5.1)**	**(2.4)**	**(0.2)**	**(0.1)**

It must be stressed that the relative order suggested above is based on a limited data set. Consequently, the level of confidence in the profile must be moderate at best. The lack of data makes it difficult to determine if the differences in extra-hepatic AHH activity listed above are in fact real, or if the levels in lung, kidney, testis and skin are approximately the same. The order of magnitude difference between the AHH activity levels in intestine and spleen and the other extra-hepatic tissues likely represents a real difference in AHH activity. Given the uncertainty surrounding the relative ranking of extra-hepatic tissue a more appropriate tissue profile may be;

Liver >> Lung = Kidney = Testis = Skin > Intestine = Spleen

D.3.2.1.2 Cytochrome P-450 Tissue Profiles in Wistar Rats

Cytochrome P-450 tissue profile data in Wistar rats is very limited. Only three studies were identified as having adequate data (JSES90,JGNO84,JVIZ80).

Total cytochrome P-450 concentration tissue profiles are similar in control Wistar and S.D. rats. Table D.3.2-9 summarizes the data available for Wistar rats.

Table D.3.2-9: Tissue Profiles of Total Cytochrome P-450 Concentrations in Wistar Rats

	Tissue ([P-450t] pmoles/mg protein)[1]			
Prep.[2]	Liver	Lung	Kidney	Ref
Microsomes	722	38 (5.3)[3]	119 (16.5)	JSES90
Microsomes	1000		128 (12.8)	JGNO84
Average		5.3	14.7	

1) Cytochrome P-450 concentration = pmoles P-450/mg microsomal protein.

2) Tissue Preparation.

3) Number in parenthesis represent % [P-450t] in hepatic tissue.

From the data in table D.3.2-9 the total cytochrome P-450 tissue profile would appear to be the following.

Liver >> Kidney > Lung
(100) (14.7) (5.3)

This agrees with the order established in S.D. rats (see Section D.3.2.1.1). In Wistar rats however, the apparent difference between kidney and lung cytochrome P-450t concentrations is greater than that found in S.D. rats. The limited quantity of data makes it difficult to assess the significance of this observation. The patterns are sufficiently similar within expected experimental limits to consider the two strains to be equivalent. Therefore, a tissue profile for total cytochrome P-450 concentrations in both Wistar and S.D. rats can be suggested as follows.

Liver >> Intestine > Kidney > Testis > Lung

It should be noted that the order suggested above is developed from a very limited data set. Further research may result in an altered tissue profile.

Tissue profiles of AHH activity are summarized in table D.3.2-10. Only two studies were judged to be adequate. As a result, conclusions based on this data must be used with caution.

Table D.3.2-10: Tissue Profiles of AHH Activity in Control Wistar Rats

	Tissue (AHH Activity = pmoles/min/mg)				
Prep.	Liver	Lung	Kidney	Epidermis	Ref
Microsomes	295±43	4.8±1.2 (1.6)	2.9±1.2 (0.98)		JGNO84
Microsomes	9±1.5			0.9±0.2 (10)	JVIZ80

The hepatic AHH activity reported by Gnojkowski *et al.* is similar to that reported by others in S.D. rats (see Section D.3.2.1.1.1). Therefore the confidence in the data must be higher than that in the values reported by Vizethum *et al.*. The hepatic AHH activity reported by Vizethum *et al.* (JVIZ80) is well below that which would be expected based on reported data in S.D. rats (see table D.3.2-4). These levels more closely resemble those found in neonatal S.D. rats (see table D.3.2-6). This suggests that the

data presented by Vizethum *et al.* (JVIZ80) is less reliable than that of Gnojkowski *et al.* and must be used with caution. Because it is unclear why the hepatic AHH activity reported by Vizethum *et al.* are low, it is difficult to determine the validity of the hepatic/epidermal AHH activity ratio. However it would appear that in the adult rat, as in the neonate, epidermal AHH levels do not exceed ten percent of those observed in hepatic tissue.

In Wistar rats, as in S.D. rats, lung AHH activity is greater than that found in kidney. Furthermore the lung/kidney percent hepatic activity ratios in Wistar and S.D. rats are very similar (Wistar [1.6/0.98 = 1.6]; S.D. [8.9/5.1 = 1.7]) This suggests that the activity in the lung is actually greater than that found in the kidney. Therefore, the AHH activity profile in both S.D. and Wistar rats is suggested to be the following.

Liver >> Lung > Kidney > Testis = Skin > Intestine

The similarity between the lung/kidney ratio in both rat strains increases the confidence that the levels in lung are indeed greater than those in the kidney. The remainder of the ranking is subject to the same cautions as those outlined for AHH activity tissue profiles in the adult male S.D. rat (see Section D.3.2.1.1.1)

D.3.2.1.3 Effect of Induction of Cytochrome P-450c Tissue Profiles

All available data for hepatic and skin tissue in neonatal S.D. rats indicates the cytochrome P-450c enzyme activity levels in skin (epidermis, dermis and whole skin) range between two and eight percent of those found in control hepatic tissue (see Section D.3.2.1.1.2). Topical application of cytochrome P-450c inducers (3-MC and Ac 1254) increased cytochrome P-450c associated enzymes activity in both hepatic and skin tissue. However, the level of induction in skin is 1.5 to 2 fold greater than that see in the liver (see table D.3.2-11).

Aryl hydrocarbon hydroxylase and ECOD activities in epidermal tissue is marginally higher than in dermal tissue in both control and induced animals. However, the increase in enzyme activity upon induction is slightly higher in dermal tissue. The significance of this is unclear. The lack of a pronounced difference between the two tissues suggests that the two can be considered equal until data indicating otherwise is available.

In adult rats, hepatic cytochrome P-450c associated enzyme activities are greater than those found in extra-hepatic tissue, both in control and induced animals (see table D.3.2-12). In hepatic tissue, treatment with either 3-MC or Ac 1254 produced a 21 and a 24 to 27 fold increase in AHH and EROD activities respectively. Induction in lung and kidney resulted in greater increases in AHH and EROD activity than that seen in hepatic tissue.

In the lung, AHH activity rose from 0.1 percent of hepatic activity in control animals to 1.1 percent of hepatic activity in animals treated with 3-MC. In renal tissue AHH activity rose from 0.43 to 4.3 percent of that found in hepatic tissue when animals were treated with inducer In pancreatic and epidermal tissue, induction resulted in an apparent decrease in AHH activity. Enzyme activity in the pancreas dropped from 0.22 percent to 0.05 percent of hepatic AHH activity. In epidermal tissue, relative activity levels dropped from 10 percent of hepatic activity in control animals to 2.2 percent in animals treated with 3-MC. The reason for this is unclear. There is insufficient data to further analyze the significance of these observations.

The decrease in epidermal AHH activity relative to hepatic activity in induced adult rats is contrary to that observed in neonatal rats. This may be explained in part by differences in induction protocols. Neonatal rats were treated topically with inducer, while the adult rats were treated with i.p. injections. In the rat there is insufficient data to determine if the different routes of treatment were responsible for the differences in the levels of induction. However, studies which have looked at AHH induction in the epidermal tissue of mice treated either by i.p. injection or by topical application have shown that the levels of AHH induction in epidermal tissue are lower in animals treated by i.p. injection than in those treated topically with inducer (JWEI75).

Table D.3.2-11: Induction of Skin and Hepatic AHH, EROD and ECOD in Neonatal Sprague-Dawley Rats

		AHH[1]		ECOD[1]		EROD[1]		
Inducer	**Tissue**	**Control**	**Induced**	**Control**	**Induced**	**Control**	**Induced**	**Ref**
	Liver	22±2						JKHA89
3-MC[2]	Epidermis	1.35±0.11	17.3±6.3 (12.8[3])	2.1±0.14	22.5±2.5 (10.4)	0.66±0.05	2.6±0.2 (3.9)	JKHA89
Ac 1254[4]	Liver	45.5	363 (7.98)	35.2	230 (6.5)			JBIC82
Ac 1254[4]	Whole Skin	1.0	13.3 (13.3)	0.83	8.4 (10.1)			JBIC82
Ac 1254[4]	Dermis	1.0	16.4 (16.4)	0.75	10.2 (13.6)			JBIC82
Ac 1254[4]	Epidermis	1.3	18.3 (14.3)	0.93	12.8 (13.1)			JBIC82
3-MC[4]	Whole Skin	2.3	26.2 (11.5)					JBIC83
Ac 1254[4]	Whole Skin	2.3	36.2 (15.9)					JBIC83

1 All enzyme activities expressed as pmoles product formed/minute/mg microsomal protein.

2 Induction treatment: Dose 40 mg/Kg 24 hours prior to sacrifice.

3 Numbers in parenthesis are fold induction over control.

4 Induction treatment: Dose 100 mg/Kg 24 hours prior to sacrifice.

Table D.3.2-12: Induction of Hepatic and Extra-hepatic AHH and EROD Activities in Adult Rats

		AHH[1]		EROD[1]		
Inducer	Tissue	Control	Induced	Control	Induced	Ref
3-MC[2]	Liver			54±12	1317±159 (24)[3]	JSTE87
Ac 1254[4]	Liver			54±12	1478±287 (27)	JSTE87
PB	Liver			54±12	67±8 (1.2)	JSTE87
3-MC[5]	Liver	39.9	856 (21.5)			JWIL90
	Lung	0.12	9.4 (78.3)			JWIL90
	Kidney	0.16	37.2 (233)			JWIL90
	Pancreas	0.09	0.47 (4.2)			JWIL90
3-MC[5]	Liver	9±1.5	187 (21)			JVIZ80
	Epidermis	0.9±0.2	4.2 (4.7)			JVIZ80

1 All enzyme activities expressed as pmoles product formed/minute/mg microsomal protein.

2 Induction treatment: Dose 40 mg/Kg via intraperitoneal (i.p.) injection 72, 48 and 24 hours prior to sacrifice.

3 Numbers in parenthesis are fold induction over control.

4 Induction treatment: Dose 500 mg/Kg single i.p. injection 5 days prior to sacrifice.

5 Induction treatment: 40 mg/Kg two i.p. injections 48 and 24 hours prior to sacrifice.

From the data presented in table D.3.2-12 a tissue profile of AHH activity can be suggested for rats treated with 3-MC. The suggested order is as follows.

Liver >> Kidney > Epidermis > Lung > Pancreas

(100) (4.3) (2.2) (1.1) (0.05)

Again it must be stressed that this order is based on a very limited data set. Therefore, all conclusions must be viewed with caution. Nevertheless the data suggest that induction has a significant effect on the potential of a number of extra-hepatic tissues to metabolize PAH. This is of particular importance as many PAH are able to act as reasonable inducers of AHH, ECOD and EROD activities (see Section D.2).

D.3.2.2 Cytochrome P-450 Tissue Profiles in Other Species

Cytochrome P-450 tissue profile data in species other than the rat is extremely limited. Table D.3.2-13 summarizes the available data. The majority of the information reports AHH activity in the liver, lung and skin in various mouse strains. One report provides data on EROD activity in the liver and testis of the Rhesus monkey and testis of the human (JDIP90). Unfortunately, the report does not contain human hepatic EROD levels. Therefore, it is difficult to compare human data with that from other species. As the bulk of the available data examines tissue profiles in the mouse, this section will focus only on this data.

Table D.3.2-13: Summary of Suitable tissue Profile Data From Species Other Than Rat

Species/Strain	Activity	Tissue	Reference
Mouse CD-1	AHH	Liver, Whole Skin	JPOH76
Mouse C57BL/6	AHH	Liver, Lung, Epidermis	JAKI76
Mouse ICR Swiss	AHH	Liver, Lung, Epidermis	JAKI76
Mouse Swiss	AHH	Liver, Lung, Whole Skin	JWEI75
Rhesus Monkey	EROD	Liver, Testis	JDIB91
Mouse CD-1	EROD	Liver, Testis	JDIB91

Table D.3.2-14 summarizes the available tissue profile data for AHH activity in the mouse. The paucity of adequate data makes a detailed analysis difficult. As can be seen from the data in table D.3.2-14, there is a large variability in the reported levels of AHH activity in control tissues. Therefore it is necessary to compare the data on a relative basis. From the data presented, it appears that in ICR Swiss and C57BL/6 mice AHH levels are 9.75 and 4.25 fold higher in the skin than in the lung, respectively. Therefore, the tissue profile suggested from this data would be the following.

Liver >> Skin > Lung

This order is markedly different from that in the rat where AHH levels in the lung are approximately 3.7 fold greater than in the skin (see Section D.3.2.1.1.2). It is difficult to assess if this represents a real difference between the two species or if it reflects normal fluctuation in experimental determination. The AHH activity tissue profile for the mouse presented above is based on a single study. Therefore, confidence in the conclusions must be accordingly low. The data from Pohl *et al* (JPOH76) which shows whole skin AHH activity at 2.3 percent of that found in the liver is in close agreement with that reported for the rat. Rat skin AHH activity levels are approximately 2.4 percent of hepatic levels (see Section D.3.2.1.1.2). The similarity between the mouse skin data reported by Pohl *et al.* (JPOH76) and that reported for the rat (Section D.3.2.1.1.2) wold suggest that tissue profiles in the mouse could be expected to be similar to those found in the rat. However, the available data does not make it possible to determine whether skin AHH levels are higher than those in the lung as is suggested by the work of Akin *et al.* (JAKI76). It is also not possible to determine if tissue profiles in the mouse are similar to those found in the rat as is suggested by the work of Pohl *et al.* (JPOH76). It does however, appear certain that AHH activity in extra-hepatic tissue is less than 12 percent of hepatic levels. This agrees reasonably well with the data reported for rats (see previous section).

D.3.2.3 Cytochrome P-450 Tissue Profiles: Summary of Conclusions

The conclusions drawn from the consideration of cytochrome P-450 tissue profiles in rats and other species are summarized below. It must be stressed that the lack of adequate data means that the level of confidence in these conclusions must be low. All of the conclusions discussed herein would benefit from larger data sets which may confirm or disprove the conclusions presented.

Table D.3.2-14: **AHH activity Tissue Profile In Control Mice**

	Liver		Lung		Whole Skin		Epidermis		
Strain	Activity[1]	% Liver[2]	Activity	% Liver	Activity	%Liver	Activity	% Liver	Reference
CD-1	1310	100			30	2.3			JPHO76
C57BL/6	70.2	100	1.5	2.6			7.8	11.1	JAKI76
ICR Swiss	80.8	100	<1	1.2			9.5	11.7	JAKI76
NIH Swiss	187.2[3]	100	15.1	8.1	17.0	9.1			JWEI75

1 Enzyme Activities expressed as pmoles product formed/minute/mg microsomal protein

2 Percent of hepatic AHH activity

3 This data is for mice induced with BAA. In addition, skin AHH activity was induced with topical application. Liver and Lung AHH activities were induced with i.p. injection. Therefore, the data were deemed unusable.

D.3.2.3.1 Cytochrome P-450 Tissue Profiles in Rats: Summary

There is limited data available on which to establish reliable tissue profiles for cytochrome P-450 levels and cytochrome P-450c associated enzyme activities. There is sufficient data to suggest preliminary tissue profiles for total cytochrome P-450 concentrations and AHH activity. There is not enough information to determine tissue profiles for either ECOD or EROD activities in the rat. The majority of the data utilized in deriving the tissue profiles shown below, was taken from S.D. rats (both neonate and adult animals) (see Section D.3.2.1.1). Limited data was also available for Wistar rats (see Section D.3.2.1.2). Although the data set for Wistar rats was smaller than that for S.D. rats, the tissue profile trends were sufficiently similar to those in the S.D. strain that the two strains could be considered equivalent; ie. strain differences were taken to be insignificant. A more detailed discussion can be found in Sections D.3.2.1.1 and D.3.2.1.2.

The tissue profile for total cytochrome P-450 concentration, derived from data for adult male S.D. rats (Section D.3.2.1.1.1) and Wistar rats (Section D.3.2.1.2) is suggested to be the following.

Liver	**>>**	**Intestine**	**>**	**Kidney**	**>**	**Testis**	**≈**	**Lung**
(100)		**(11)**		**(9.2)**		**(6.4)**		**(5.9)**

Where the numbers in parenthesis represent the total cytochrome P-450 concentration as a percent of that in hepatic tissue.

There is insufficient data to establish if the differences in total cytochrome P-450 concentration between extra-hepatic tissue represent real differences or if they are due to experimental variation. Therefore, the confidence in the suggested order must be low.

The tissue profile for AHH activity was derived from neonatal and adult S.D. rats (see Section D.3.2.1.1) and adult Wistar rats (see Section D.3.2.1.2). The tissue profile was found to be somewhat different from that for the total cytochrome P-450 concentrations. The AHH activity tissue profile in rats is suggested as follows.

Liver	**>>**	**Lung**	**>**	**Kidney**	**>**	**Testis**	**≈**	**Skin**	**>**	**Intestine**
(100)		**(8.9)**		**(5.1)**		**(2.4)**		**(2.4)**		**(0.2)**

It is interesting to note that lung and intestine have changed relative positions in the AHH activity profile when compared to the total cytochrome P-450 profile. Lung, which ranked lower than all other extra-hepatic tissues in total cytochrome P-450 concentrations has the highest level of cytochrome P-450c associated AHH activity in extra-hepatic tissue. Intestine, which had the highest level of total cytochrome P-450 in extra-hepatic tissue had the lowest level of AHH activity (see Section D.3.2.1.1). It should be remembered that cytochrome P-450c is the isozyme primarily responsible for the metabolism and activation of PAH (see Sections D.1 and D.2). While there is only an apparent two fold difference between intestine and lung total cytochrome P-450 concentrations, there is a forty four fold difference in the levels of cytochrome P-450c. This marked difference in potential for PAH metabolism between the two tissues may help to explain, in part, the observed differences in sensitivity to PAH when administered via inhalation or ingestion (see Main Document).

The level of confidence in the tissue profile for AHH activity is greater than that for the total cytochrome P-450 concentration profile. There are two main reasons for this;

i) The levels of AHH activity in the intestine are at least ten fold lower than that found in other extra-hepatic tissues. This difference is greater than would be attributable to experimental variation. Variation in reported activities in a single tissue typically range between one to six fold (see data tables in Section D.3.2.1.1 and Section D.3.2.1.2).

ii) The ratio between the relative levels of AHH activity in lung and kidney (%Hepatic AHH in Lung/% hepatic AHH in Kidney) is essentially the same for Wistar (1.6) and S.D. (1.7) rats.

This suggests that the difference between these tissues can be considered as real and not due to experimental variation. The levels of AHH activity in skin and testis are equal and both are more than two fold lower than levels found in kidney. Although this would appear significant, there is insufficient data to establish conclusively that the levels of AHH activity in the testis and skin are lower than those found in the kidney. Consequently, the ranking suggested above must be used with caution.

D.3.2.3.2 Effects of Induction on Cytochrome P-450 Tissue Profiles: Summary

Treatment of rats with either 3-MC or Ac 1254 increases the levels of cytochrome P-450c associated enzyme activities in hepatic and extrahepatic tissue (see Section D.3.2.1.3). In control animals and those treated with inducers, the levels of AHH activity are highest in the liver. However, the increase in AHH activity in response to induction are higher in extra-hepatic tissue than in the liver. The skin data was derived from normalized whole skin/liver ratios in table D.3.2-11. The levels of induction in extra-hepatic tissue, particularly kidney, results in an AHH tissue profile which is different form that seen in control animals. In animals treated with 3-MC, the tissue profile would appear to be the following.

Liver >>	Kidney >	Epidermis >	Lung
(100)	(4.3)	(2.2)	(1.1)

The large increase in cytochrome P-450c associated enzyme activity in extra-hepatic tissues may help to explain the increased sensitivity to the tumorigenic effects of PAH observed in extra-hepatic tissue (see Appendix A: Tumorigenicity of B[a]P and 3-MC in Rodents). However, much more data is required to explain this further. The limited data means that confidence in the induction tissue profile is limited. However, the magnitudes of the differences in the levels of induction suggest that induction of AHH activity in extra-hepatic tissue may play a major role in tissue sensitivity.

D.3.2.3.3 Cytochrome P-450 Tissue Profiles in Other Species: Summary

There is insufficient data of adequate quality on cytochrome P-450 tissue profiles in species other than the rat to draw any conclusions. Two studies are available which examine AHH activity in liver and skin. One of these also reports AHH levels in lung tissue (see Section D.3.2.2). The study which reports AHH activity in liver, lung and skin shows that, in mice, AHH activity is greater in the skin than in the lung. This is contrary to the order determined for rats; where the highest levels of AHH activity in extra-hepatic tissue are found in the lung. The second study which reported AHH activity only in the liver and whole skin showed that the relative levels of AHH activity in whole skin was 2.3 percent of that found in hepatic tissue. This is very similar to that reported in the rat; where whole skin AHH activity was 2.4 percent of that found in the liver. Unfortunately, as this latter report failed to present any data for AHH activity in mouse lung, it is impossible to determine if the relationship between skin and lung is valid. Further comparison between mouse and rat is not possible. However, it does appear reasonably certain that AHH activity in mouse extra-hepatic tissue does not greatly exceed ten percent of that found in hepatic tissue (see Section D.3.2.2)

D.3.3 Tissue Profiles for Epoxide Hydrolase

Epoxide hydrolase (E.H.) activity has been studied in a number of tissues from a variety of species. Rat and mouse are the most commonly studied species, but data are available for guinea pig, rabbit, cat pig, Rhesus monkey and human. Epoxide hydrolase has been detected in many tissues including; liver, lung, kidney, skin, intestine, spleen, testis and muscle (JOES77). Because E.H. is responsible for the conversion of epoxides to dihydrodiols differences in E.H. levels between tissues, strains and species, may provide a partial explanation for the observed differences in sensitivity to PAH. The following sections examine the available data on the tissue distribution of E.H. in rats and other species.

D.3.3.1 Epoxide Hydrolase Tissue Profiles In Rats

There are a limited number of studies which have investigated E.H. activity in more than one tissue. The studies which were found to contain adequate data are listed in table D.3.3-1. The substrates used to monitor E.H. activity and the tissues tested by each study are also provided in table D.3.3-1. The majority of the studies listed have examined E.H. tissue activity in the S.D. rat. One study examined E.H. activity in the Fisher 344 rat strain (JDIB91).

Table D.3.3-1: Summary of Acceptable Epoxide Hydrolase Data in Multiple Tissues From Control Rats

Strains/Sex	Substrate	Tissue	Ref
S.D. (M)	B[a]P-4,5-epoxide	Liver, Whole Skin	JBEN76
S.D. (M)	B[a]P-4,5-epoxide	Liver, Lung, Kidney	JSCH78
S.D. (M)	B[a]P-4,5-epoxide	Liver	JWAL78
S.D. (M)	B[a]P-4,5-epoxide	Liver, Lung, Kidney, Epidermis Intestine, Spleen, Testis	JOES77
S.D. (M)	B[a]P-4,5-epoxide	Whole Skin, Dermis, Epidermis	JBIC82
Fisher 344	*cis*-stilbene oxide	Liver, Testis	JDIB91

Epoxide hydrolase activities in hepatic and extra-hepatic tissue are summarized in table D.3.3-2. All studies which use S.D. rats measured E.H. activity using B[a]P-4,5-epoxide as substrate. DiBiasio *et al.* reported E.H. activity in liver and testis of Fisher 344 rats using *cis*-stilbene oxide as the E.H. substrate (JDIB91). Therefore direct comparisons between Fisher 344 and S.D. rats is difficult. However, comparisons of E.H. activity in extra-hepatic tissues relative to hepatic E.H. activity levels are possible.

As can be seen from the data in table D.3.3-2, there is little variation in E.H. activity between reports. Thus, the level of confidence in the tissue profile suggested below is relatively high. The data for whole skin, dermis and epidermis in the neonate S.D. rat are sufficiently similar that they may be considered as being equal. Therefore, the data for whole skin was used in the development of the tissue profile. From the data presented in table D.3.3-2, a tissue profile for E.H. activity in control rats can be suggested as follows.

Liver	**>>**	**Testis**	**>**	**Kidney**	**>**	**Lung**	**>**	**Spleen**	**=**	**Intestine**	**=**	**Skin**
(100)		**(22.1)**		**(11)**		**(5.3)**		**(2.1)**		**(2.0)**		**(1.8)**

D.3.3.2 Effect of Induction on E.H. Tissue Profiles

There appears to be only one report which examines the effect of cytochrome P-450 inducers on E.H. tissue profiles in rats (JOES77). Oesch *et al.* monitored the effect that i.p. treatment with Ac 1254 had on E.H. tissue profiles in adult male S.D. rats. The data are summarized in table D.3.3-3. The data show that treatment with Ac 1254 has little effect on E.H. activity in extra-hepatic tissue and results in only a 1.7 fold increase in hepatic E.H. activity. Therefore, induction lowers the levels of E.H. in extra-hepatic tissue relative to liver. The tissue profile order, however does not change from that noted in control animals. The most notable change in relative E.H. activity is seen in the testis which falls from 22.1 percent of the hepatic activity to 9.2 percent. There is insufficient data to determine the significance of this observation.

D.3.3.3 Epoxide Hydrolase Tissue Profiles in Other Species

Data relating to E.H. tissue profiles in species other than rat appears to be limited to a very small number of reports. Unfortunately, comparison between these reports is hindered by the lack of a consistent choice of E.H. substrate between reports. A summary of the data judged to be adequate is provided in table D.3.3-4.

Analysis of E.H. tissue profiles in the mouse presents a problem as only one report by Oesch *et al.* (JOES77) has used B[a]P-4,5-epoxide as a substrate to monitor E.H. activity in multiple tissues. This work indicated that E.H. activity was highest in the testis followed by the liver. Oesch suggests that in most species, the tissue distribution of E.H. activity is similar to that found in the rat, although no data is provided to support this. In addition, Oesch (JOES77) states that NMRI mice which show the highest levels of E.H. activity in the testis, are an exception to this order. Also, in NMRI mice, E.H. activity in the lung is greater than that found in the kidney (see table D.3.3-4). This is contrary to that found in the rat (see Section D.3.3.1). DiBiasio *et al.* (JDIB91) found that in CD-1 mice, E.H. activity in the testis was greater than that found in the liver (see table D.3.3-4), and Waechter *et al* (JWAE88) showed that lung E.H. activity was greater than that in the kidney (see table D.3.3-4). In this latter study however, E.H. activity in the liver was greater than that reported in the testis (JWAE88) (see table D.3.3-4). Direct comparison between these studies is hampered by the lack of a common substrate (see table D.3.3-4). It would appear however, that NMRI mice are not unique in having a higher level of E.H. activity in the testis than in the liver.

For mice, the apparently conflicting data regarding the E.H. tissue profile is difficult to attribute to either strain or substrate differences. Multiple tissue analysis in more than one strain, using a common E.H. substrate would provide data useful in addressing this issue. As a result of the conflicting data, the E.H. tissue profile was developed solely from the work of Oesch *et al.* (JOES77). This work was chosen for two principle reasons; Firstly, it provided the widest range of tissue analysis and; Secondly, it use B[a]P-4,5-epoxide as the E.H. substrate. The choice of substrate will facilitate comparison with rat E.H. tissue profile data by removing concerns over substrate. From this data, the tissue profile for E.H. activity in the mouse is suggested to be the following.

Testis	**>>**	**Liver**	**>**	**Lung**	**>**	**Skin**	**>**	**Kidney**	**>**	**Intestine**	
(243)		**(100)**		**(48)**		**(19)**		**(19)**		**(3)**	

The only other species for which adequate E.H. tissue profile data could be found was the rabbit (see table D.3.3-4) (JWAL78). The authors of this report state that there is a five percent variation in the standards error around each point. Without further data it is difficult to assess the validity of the differences in the extra-hepatic tissue levels. It is clear that the highest levels of E.H. are in the liver (JWAL78). The data shown in table D.3.3.-4 suggest the following E.H. profile.

Liver	**>>**	**Testis**	**>**	**Kidney**	**>**	**Lung**
(100)		**(15)**		**(10.6)**		**(8.1)**

While the significance of the difference in E.H. activity between extra-hepatic tissues is difficult to assess, the order outlined above is similar to that noted for the rat (see Section D.3.3.1).

D.3.3.4 Epoxide Hydrolase Tissue Profiles; Summary of Conclusions

The conclusions drawn from the consideration of epoxide hydrolase tissue profiles in rats and other species are summarized below. The paucity of data for species other than the rat reduces the confidence in the conclusions presented. All of the conclusions discussed herein would benefit from larger data sets which would confirm or refute the conclusions presented.

D.3.3.4.1 Epoxide Hydrolase Tissue Profiles in Rats; Summary

There is reasonable data available for determining E.H. tissue profiles in S.D. rats. The small variability in cited E.H. levels in a single tissue (see table D.3.3-2) increases the confidence in the tissue profile suggested from this data. The suggested E.H. tissue profile is the following (numbers in parenthesis are percent hepatic activity).

Liver	**>>**	**Testis**	**>**	**Kidney**	**>**	**Lung**	**>**	**Spleen**	**=**	**Intestine**	**=**	**Skin**
(100)		**(22.1)**		**(11)**		**(5.3)**		**(2.1)**		**(2.0)**		**(1.8)**

The lack of data for other strains of rats makes it impossible to determine what, if any, strain differences exist.

Table D.3.3-2: Epoxide Hydrolase Tissue Profiles in Control Rats

		Tissue (E.H. Activity = nmoles product formed/minute/mg microsomal protein)									
Strain	**Prep**	**Liver**	**Lung**	**Kidney**	**Whole Skin**	**Epidermis**	**Dermis**	**Intestine**	**Spleen**	**Testis**	**Ref**
S.D. (M)	Microsomes	7.48±0.81			0.12±0.02						JBEN76
S.D. (M)	Microsomes	6.65±0.76	0.33±0.05	0.75±0.10							JSCH78
S.D. (M)	Microsomes	6.1									JWAL78
S.D. (M)	Microsomes	6.39±0.64	0.36±0.05	0.71±0.12		0.14±0.02		0.13±0.03	0.14±0.02	1.47±0.25	JOES77
S.D. Neonate	Microsomes				0.12±0.01	0.16±0.01	0.14±0.01				JBIC82
Fisher 344 (M)	Microsomes	8.86[1]								3.9	JDIB91
Mean ±Standard Error		6.66±0.59	0.35±0.02	0.73±0.03	0.12±0.0	0.15±0.01	0.14	0.13	0.14	1.47	
% Hepatic Activities		100	5.3	11	1.8	2.3	2.1	2.0	2.1	22.1	

1 Data excluded from calculation due to different strain and substrate (see text).

Table D.3.3-3: Effects of Ac 1254 Treatment on Epoxide Hydrolase Tissue Profiles

		Tissue (E.H. Activity = nmoles product formed/minute/mg microsomal protein)							
Strain	Treatment	Liver	Lung	Kidney	Epidermis	Intestine	Spleen	Testis	Ref
S.D. (M)	Control	6.39±0.64	0.36±0.05	0.71±0.12	0.14±0.02	0.13±0.03	0.14±0.02	1.47±0.25	JOES77
S.D. (M)	Ac 1254[1]	11.36±0.71	0.34	0.82±0.02	0.14	0.13	0.14	1.05	JOES77
Fold Induction		1.78	0.94	1.15	1.00	1.00	1.00	0.71	
% Hepatic Activities		100	3.0	7.2	1.2	1.1	1.1	9.2	

1 Induction treatment = single i.p. injection of Ac 1254 (300 mg/kg) 5 days prior to sacrifice.

Table D.3.3-4: Epoxide Hydrolase Tissue Profiles in Other Species

		Tissue (E.H. Activity = nmoles product formed/minute/mg microsomal protein)						
Species/Strain	Substrate	Liver	Lung	Kidney	Whole Skin	Intestine	Testis	Ref
Mouse (NMRI)	B[a]P-4,5-epoxide	0.90±0.1	0.43±0.15	0.07±0.01	0.17±0.02	0.03	2.19±0.40	JOES77
Mouse (DBA)	Styrene Oxide	1.76±0.3	0.46±0.07	0.13±0.06			1.0±0.1	JWAE88
Mouse (CD-1)	*Cis*-Stilbene Oxide	7.73					12.22	JDIB91
Rabbit (NZW)[1]	B[a]P-4,5-epoxide	16	1.3	1.7			2.4	JWAL78

1 New Zealand White

D.3.3.4.2 Effects of Induction of Epoxide Hydrolase Tissue Profiles; Summary

Treatment of rats with a single i.p. injection of Ac 1254 (300 mg/Kg) had no appreciable effect on E.H. activity in extra-hepatic tissue, and caused only a marginal (1.7 fold) increase in hepatic E.H. activity (see Section D.3.3.2).

D.3.3.4.3 Epoxide Hydrolase Tissue Profiles in Other Species; Summary

Limited data is available for species other than the rat (see Section D.3.3.3). In the mouse, the apparent tissue profile is suggested to be the following.

Testis	**>>**	**Liver**	**>**	**Lung**	**>**	**Skin**	**>**	**Kidney**	**>**	**Intestine**
(243)		**(100)**		**(48)**		**(19)**		**(19)**		**(3)**

In the rabbit, the apparent tissue profile is similar to that in the Sprague-Dawley rat, and is suggested to be the following.

Liver	**>>**	**Testis**	**>**	**Kidney**	**>**	**Lung**
(100)		**(15)**		**(10.6)**		**(8.1)**

For both of the tissue profiles presented above, the numbers in parenthesis represent the percent of hepatic E.H. activity found in each tissue. The lack of data for both of the tissue profiles shown above dictates that the levels of confidence in these profiles must be low.

D.3.4 Tissue Profiles For Glutathione-S-Transferases

Glutathione-S-transferases (GST) have been found in almost all rat tissues examined (JCOL90). While studies of GST have been conducted in numerous species and tissues, there exists little data which is adequate for developing tissue profiles. There are two principle reasons for this; Firstly, most studies focus on the hepatic system of the species in question and rarely report data in other tissues; Secondly, much of the GST work available in the literature examines GST isozyme distribution. Therefore when extra-hepatic tissues is examined, data on more than one tissue is seldom available. What work does exist suggests that GST levels are higher in the liver than in other organs (JCOL90). The lack of adequate data makes developing tissue profiles for any species extremely difficult. The following sections examine the available tissue profile data.

D.3.4.1 Glutathione-S-Transferase Tissue Profiles in Rats

Although there is ample data on rat hepatic GST and the various GST isozymes, there exists a very limited number of studies which contain data suitable for developing tissue profiles. Table D.3.4-1 summarizes the available data.

Table D.3.4-1: Glutathione-S-Transferase Activity Tissue Profiles in Control Rats

			Tissue (GST Activity = nmoles/minute/mg[1])				
Strain/Sex	**Prep**	**Substrate**	**Liver**	**Lung**	**Kidney**	**Testis**	**Ref**
S.D.	105,000 x g[2]	CDNB	352±104		199±19 (56)		JTAR91
Wistar	S9[3]	CDNB[4]	1171±138	59.3±8.6	185.6±23.3		JGNO84
Fisher 344	105,000 x g	stil-ox[5]	131.3			101.5 (77)	JDIB91

1 GST Activity = nmoles product formed/minute mg cytosolic protein.

2 Cytosol derived from 105,000 x g 60 minute supernatant from microsomal preparations.

3 Cytosol derived from 10,000 x g - 10 minute supernatant from initial tissue homogenization.

4 1-chloro-2,4-dinitrobenzene

5 *cis*-stilbene oxide

There are two types of cytosolic preparations used as a source of GST. The first labelled "*S9*" is the supernatant derived from a 10 to 20 minutes centrifugation at approximately 10,000 x g of the initial tissue homogenate. The second, identified as "*105,000 x g*" is the supernatant derived from a greater than 60 minute centrifugation at 105,000 x g of the S9 fraction. The data in table D.3.4-1 suggest that there is a difference between the two preparations. Glutathione-S-transferase activity would appear to be higher in the S9 cytosol than in the 105,00 x g cytosol. The data are more complete for S9 cytosolic preparations where data is available for liver, lung and kidney. Therefore this data was given preference over that from the 105,00 x g derived cytosol. While this second set of data reports GST levels in Kidney (JTAR91) and testis (JDIB91), the two studies used different substrates to monitor GST activity. Therefore, direct comparison between the two would be uncertain. For these reasons, preference was given to the S9 data set. The suggested tissue profile developed from the S9 cytosol data is the following.

Liver	>>	**Kidney**	>	**Lung**
(100)		**(15.8)**		**(5.1)**

D.3.4.2 Effects of Induction on Glutathione-S-Transferase Tissue Profiles

There is limited induction data available for GST activity in the liver, from rats and rabbits. The data are summarized in table D.3.4-2. A direct comparison between the two species id difficult as the tissue preparations differ. As can be seen from the data in Section D.3.4.1, the tissue preparation appears to have a marked effect on hepatic GST activity (see table D.3.4-1). Rats treated with cytochrome P-450 inducers, show increases in hepatic GST levels. Inducers specific for cytochrome P-450c (3-MC) have the smallest effect while mixed type inducers (those which effect both cytochrome P-450b and P-450c) have the greatest effect. In the rabbit, treatment with both PB (cytochrome P-450b specific) and 3-MC caused an apparent reduction in GST activity. There is insufficient data to allow further analysis of the effects of inducers on GST levels.

Table D.3.4-2: Effect of Inducers on Hepatic Glutathione-S-transferase Activity

			Tissue (GST Activity = nmoles/minute/mg[1])				
Species	**Prep**	**Substrate**	**Control**	**PB**	**3-MC**	**Ac 1254**	**Ref**
Rat (S.D.)	Broken Cells	CDNB	1054±138	1796±278	1520±190	1870±359	JSTE87
Rabbit	105,000 x g	CDNB	15700±1500	12900±1200	13100±3300		JPRI91

D.3.4.3 Glutathione-S-Transferase Tissue Profiles in Other Species

Data suitable for developing tissue profiles in species other than the rat could not be located in the literature. One study, however, looked at hepatic GST activity in numerous strains of mice (JBOR91). Hepatic GST activity in the various strains ranged between 2750 and 5300 nmole/min/mg with a mean and standard deviation of 4244±768. These data were determined from graphical information presented in the report of Borroz *et al.* (JBOR91). Therefore, there is a reasonable degree of uncertainty associated with each data point cited in table D.3.4-3. However, from this data it would appear that strain differences are negligible.

Table D.3.4-3: Hepatic Glutathione-S-transferase Levels in Various Mouse Strains (From JBOR91)

Strain	Swiss1	CD-1	A/J	CBA/CA	BALB/c	C57BL/6	C3H	DBA/2	Mean±S.E.
Activity	3750	4750	2750	4750	4250	5300	4100	4300	4222±768

D.3.4.4 Glutathione-S-Transferase Tissue Profiles: Summary

There is insufficient data in any species to develop meaningful tissue profiles. In the rat, the only species for which a profile could be suggested, the highest levels were found in the liver, followed by kidney and lung (see Section D.3.4.1). Much more data in multiple tissues is required before tissue profiles can be suggested for this group of enzymes.

D.3.5 Tissue Profiles for UDP-Glucuronyl Transferases

An extensive literature search failed to locate data which could be used to develop tissue profiles for UDP-glucuronyl transferases (UDP-GT). However, data does exist which examines the effect of cytochrome P-450 inducers on UDP-GT levels in rat, mouse and human hepatic tissue (JBOC87). These data are summarized in table D.3.5-1. The work of Bock *et al.* (JBOC87) shows that treatment with either 3-MC or PB increases the levels of UDP-GT in hepatic tissue in all three species (see table D.3.5-1). Treatment with 3-MC causes the greatest level of induction of UDP-GT regardless of the substrate used to measure UDP-GT activity (JBOC87). In addition, treatment with 3-MC appears to cause a greater level of induction in the rat than in the mouse. This is difficult to evaluate properly as the treatment doses for the two species were not reported. An inter-species comparison shows that UDP-GT levels are highest in the rat and lowest in the human. The apparent species order can be suggested as follows.

Rat > Mouse > Human

As these results come from a single study, there is a reasonable degree of confidence in the order suggested above. However, further data would aid in establishing the validity of this conclusion.

Table D.3.5-1: Species Comparison of Hepatic UDP-Glucuronyl Transferase Activity

	Hepatic UDP-GT Activity nmoles product/min/mg microsomal Protein						
	1-Naphthol			B[a]P-3,6-quinol			
Species	Control	PB	3-MC	Control	PB	3-MC	Ref.
Rat	40±6	64±23	178±45	5.6±0.9	10.9±3.3	51.8±15.4	JBOC87
Mouse	24±4	38±6	36±3	2.9±0.1	5.6±0.3	7.0±0.1	JBOC87
Human	15.8	31.7		1.8	3.3		JBOC87

D.3.6 Tissue Profiles for Sulphotransferases

Data suitable for developing tissue profiles for sulphotransferase (ST) activity could not be found in the literature.

D.4 PAH-DNA Adduct Formation

Polynuclear aromatic hydrocarbons are metabolized to a large number of products. The majority of these are readily excreted from the body. A small percentage, however, are converted to reactive compounds that are capable of reacting with biological macromolecules. It is the binding of the reactive metabolites to cellular subcomponents, particularly DNA, that is thought to be responsible for the biological activity of these compounds (BGEA88, JJAN90).

Differences in tissue and species sensitivity to the tumorigenic effects of PAH may be related to the total number of adducts formed, or to the specific types of adducts formed, or to a combination of these two factors. The following sections examine total adduct formation and the formation of specific PAH-DNA adducts to determine if either can be correlated with observed differences in tissue and species sensitivities to PAH.

D.4.1 PAH-DNA Total Adduct Formation

This section examines total PAH-DNA adduct formation in a number of species to determine if total binding can be correlated with differences in observed tissue and species sensitivity to PAH (see Main Document). It is not concerned with the identity of the individual PAH-DNA adducts formed but rather, it examines the assumption that it is the total number of PAH-DNA adducts formed by a given PAH is one of the primary factors in determining the tumorigenic response.

D.4.1.1 PAH-DNA Total Binding Following Topical Application of PAH

The majority of the PAH-DNA total binding data available in the literature looks at adduct formation in epidermal tissue following topical application of PAH. These data are summarized in tables D.4.1-1 through D.4.1-12. Care must be taken when selecting data to insure that only studies which report total PAH-DNA binding or total adduct formation are used. Studies which fail to distinguish between total binding and total PAH-DNA binding should not be used. Tritium exchange between labelled PAH and non-adducted DNA, and a lack of distinction between deoxyribonucleosides and deoxyribonucleotides can produce erroneous results by over estimating the actual levels of binding (JBUR83, JPHI79). Unless otherwise indicated, the data used in the subsequent sections have been assembled using these selection criteria.

Table D.4.1-1: PAH-DNA Total Binding at 19 Hours Post Treatment (Available Data)

Compound	Dose (μmoles)	Strain (Sex)	Reference
B[a]P	0.10	Swiss (M)	JPHI78
		C57BL (M)	JPHI78
		DBA/2 (M)	JPHI78
	0.25*	Swiss (M)	JROJ86
	1.00	C57BL (M)	JPHI79
DMBA	0.10	Swiss (M)	JPHI78
		C57BL (M)	JPHI78
		DBA/2 (M)	JPHI78
	1.00	C57BL (M)	JPHI78
3-MC	1.00	Swiss (M)	JPHI78
		C57BL (M)	JPHI78, JPHI79
		DBA/2 (M)	JPHI78

* Data from 18 hour study not 19 hours.

Much of the selected data reports total adduct formation at either 19 or 24 hours post treatment. Although data for other time points are available, the 19 and 24 hour time points provide the largest data sets from which comparisons can be made. At 19 hours post treatment, data on the binding of a number of PAH in a number of mouse strains is available (see table D.4.1-1). At the 24 hour time point, data is available primarily for DMBA- and B[a]P-PAH binding in female SENCAR mice with limited data from other mouse strains (See table D.4.1-2). Therefore it is difficult to make direct comparisons between SENCAR mice and other mouse strains. Combining the data from the 19 and 24 hour data sets would allow a more direct comparison between SENCAR and other mouse strains. Because SENCAR mice are reported to be the strain most sensitive to the tumorigenic effects of PAH (JDIG84), comparisons of this nature may be beneficial in understanding the observed strain and species differences in sensitivity to PAH tumorigenicity. The following section examines the time course data for PAH-DNA adduct formation to determine if the 19 and 24 hour data sets can be combined in a meaningful manner.

D.4.1.1.1 PAH-DNA Total Binding: Time Course Analysis

The time-course binding of PAH metabolites to DNA has been examined for various PAH in a number of mouse strains (JDIG85, JSMO87, JPHI78, JPHI79). The largest data sets exist for DMBA and B[a]P, although binding data for BAA, 3-MC, Dibenz[a,c]anthracene (DB[a,c]A), Dibenz[a,h]anthracene (DB[a,h]A) and 7-methylbenaz[a]anthracene (7-MeBAA) have also been reported (JPHI79).

For all compounds examined, except DB[a,h]A, PAH-DNA binding reached a maximum within 24 hours. Dibenz[a,h]anthracene-DNA binding reached a maximum only after 72 hours (JPHI79). Phillips et al. have reported that the binding of B[a]P and DMBA and 3-MC is at or near maximal levels between 18 and 24 hours (JPHI79). Smolarek et al. have reported time-course binding data for B[a]P in SENCAR mice which suggests that maximal binding is reached by 12 hours after topical application of 0.2 µmoles of B[a]P (see fig 4.1-1) (JSMO87). Similar work by DiGiovanni et al. which examined binding in SENACR mice at the same dose (0.2 µmoles) showed a binding maximum at 24 hours (see fig 4.1-1) (JDIG85). Both studies failed to examine binding between 18 and 19 hours where it has been suggested that binding is at maximal levels (JPHI79). Therefore it is possible that both studies failed to detect the maximum level of binding. Consequently, it is difficult to determine the time at which maximal binding occurs.

Table D.4.1-2: PAH-DNA Total Binding at 24 Hours Post Treatment (Available Data)

Compound	Dose (µmoles)	Strain (Sex)	Reference
B[a]P	0.05	SENCAR (F)	JSMO87
	0.20	SENCAR (F)	JDIG85, JSMO87, JDIG86
		C57BL (M)	JASH82
DMBA	0.01	SENCAR (F)	JDIG83, JDIG86, JDIG86A
		NIH Swiss (M)	JDIP84
		C57BL (M)	JDIP84
	0.025	SENCAR (F)	JDIG86A
	0.5	SENCAR (F)	JDIG86A
	0.10	SENCAR (F)	JDIG86A
		NIH Swiss (M)	JDIP84
		C57BL (M)	JDIP84

Examination of DMBA-DNA binding in three different mouse strains, Swiss, C57BL and DBA/2, showed similar binding patterns in all three strains (see fig 4.1-2) (JPHI78). There are however some minor difference. The time-course binding for the DBA/2 and C57BL mouse strains show apparent DMBA-DNA binding maxima at 24 hours. In Swiss mice, the apparent maximum is achieved at 18 hours. As mean and standard deviations were not provided for the data presented in figure D.4.1-2 it is difficult to estimate the significance of the difference between the Swiss and DBA/2 and C57BL mouse strains. The degree of uncertainty surrounding the location of the binding maximum suggests that combining data derived at 19 and 24 hours would be unreliable as it is not possible to predict if the binding maximum has been reached or exceeded. Consequently, comparisons between the binding of individual PAH to DNA within a single mouse strain and comparisons of the binding of a single PAH to DNA between strains will be restricted to data taken at the same time point.

Figure D.4.1-1: Time-Course for B[a]P-DNA binding in SENCAR Mice

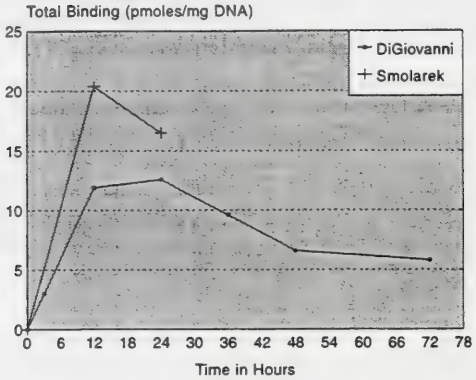
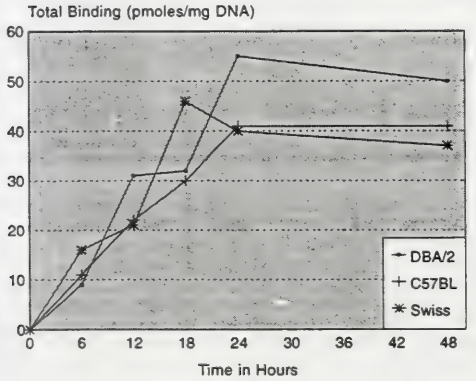


Figure D.4.1-2: Time-Course for DMBA-DNA Binding in Swiss, C57BL and DBA/2 Mice



The binding data for B[a]P and DMBA presented in figures D.4.1-1 and D.4.1-2 show a difference in the persistence of the adducts derived from B[a]P and DMBA. The time-course data for B[a]P-DNA binding in female SENCAR mice shows a drop in total adducts levels of approximately 50 percent between 24 and 48 hours (JDIG85). Over the same time period DMBA-adduct levels in Swiss, C57BL and DBA/2 mice show no appreciable change. It is possible that the apparently greater persistence of DMBA-DNA adducts compared to B[a]P-DNA adducts is, in part, responsible for the greater tumorigenic potency of DMBA (JPHI79 and others). Unfortunately, the DMBA and B[a]P-DNA binding data were not reported in the same mouse strains, therefore a direct comparison between B[a]P and DMBA is not possible. There is insufficient data available to evaluate this further.

D.4.1.1.2 PAH-DNA Total Binding: Strain and Species Comparisons

A number of authors have reported that a good correlation exists between PAH-DNA binding affinity and tumorigenic response within an individual mouse strain (JDIG84,JSLA82,JPHI79). The binding and tumorigenic potencies are reported as (JSLA82, JPHI79) follows.

DMBA > 3-MC≥B[a]P >> BAA

This is an approximate order that appears to hold for a number of mouse strains including; SENCAR, Swiss, C57BL and DBA/2 (JPHI78,JDIG84,JSMO87). The data supporting this observation are examined in section D.4.1.2.1: PAH-DNA Binding in Individual Mouse Strains.

Reports in the literature suggest the correlations of PAH-DNA binding and tumorigenic response between mouse strains are poor (JPHI78, JPHI79, JDIG84). The validity of this observation is examined in section D.4.1.2.2: PAH-DNA Binding: Inter-Strain Comparisons.

The availability of PAH-DNA binding data following topical application of PAH in species other than the mouse is limited. Section D.4.1.2.3 PAH-DNA Total Binding: Inter-Species Comparisons, examines the data to determine whether qualitative or quantitative correlations between PAH-DNA binding and tumorigenic response between species do exist.

D.4.1.1.2.1 PAH-DNA Total Binding: Individual Mouse Strains

D.4.1.1.2.1.1 PAH-DNA Total Binding in SENCAR Mice

In SENCAR mice PAH-DNA total binding in epidermal tissue is available for B[a]P and DMBA (Table D.4.1-3). The only dose which is common to both compounds is 0.05 µmoles. At this dose, DMBA binding is about 7 fold greater than that of B[a]P (see figure D.4.1-3). From the data presented in figure D.4.1-3 it would appear that as the amount of applied PAH increases, the difference in total binding between B[a]P and DMBA decreases. This may suggest that the level of adduction is a saturable process. However, there is insufficient data available to evaluate this.

Table D.4.1-3: PAH-DNA Total Binding in SENCAR Mice 24 Hours After Topical Application of PAH

Compound	Dose (µmoles)	Total Binding (pmoles/mg DNA)	Reference
B[a]P	0.05	1.6	JSMO87
B[a]P	0.20	12.4	*
DMBA	0.01	3.8	**
DMBA	0.025	6.0	JDIG86A
DMBA	0.05	11.2	JDIG86A
DMBA	0.10	16.2	JDIG86A

* Value averaged from JDIG85, JSMO87 and JDIG86 (table D.4.1-1)

** Value Averaged from JDIG83, JDIG86 and JDIG86A (table D.4.1-2)

For SENCAR mice, the order of PAH-DNA binding is the following.

DMBA > B[a]P

At the 0.05 µmole dose, there is a 7 fold difference in PAH-DNA binding for the two compounds. The data presented in figure D.4.1-3 suggests that the binding is dose dependent. Therefore, at a different dose level the magnitude of the difference in PAH-DNA binding between DMBA and B[a]P is likely to be different from that seen at the 0.05 µmole dose level.

D.4.1.1.2.1.2 PAH-DNA Total Binding in Swiss Mice

In Swiss mice, PAH-DNA binding data is available for DMBA, B[a]P and 3-MC (see Table D.4.1-4). As was seen with SENCAR mice, DMBA exhibits a higher level of PAH-DNA binding than B[a]P. However, the difference between the two appears to be only 2 to 3 fold compared to the 7 fold seen in SENCAR mice. Unfortunately, the binding data for 3-MC for Swiss mice does not share a dose common to either B[a]P or DMBA. Thus a direct comparison of total binding between all three compounds is not possible. However, by examining PAH-DNA binding data for other mouse strains in which DMBA and B[a]P have been tested at two or more doses, including 1.0 µmoles. is impossible to estimate DMBA and B[a]P-DNA binding levels in Swiss mice at the 1.0 µmole dose level.

Figure D.4.1-3: PAH-DNA Total Binding Dose-Response Curves for DMBA and B[a]P

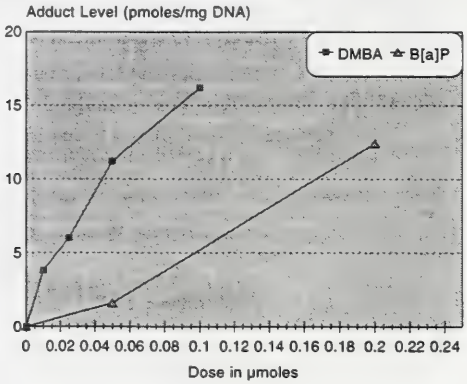


Table D.4.1-4 PAH-DNA Total Binding in Swiss Mice 19 Hours After Topical Application of PAH

Compound	Dose (µmoles)	Total Binding (pmoles/mg DNA)	Reference
B[a]P	0.10	2.6	JPHI78
B[a]P	0.25*	7.1	JROJ86
	1.0	16.6	Estimated
DMBA	0.10	7.2	JPHI79
	1.0	30.0	Estimated
3-MC	1.0	24.5	JPHI78

* Data is for 18 hours not 19

The C57BL mouse strain is the only one for which DMBA and B[a]P binding data is available at the 0.1 and 1.0 µmole dose levels (See table D.4.1-5). For DMBA, the increase between the 0.1 and 1.0 µmole doses is approximately 4 fold (table D.4.1-5) (JPHI79). For B[a]P, there is an approximate 6.4 fold increase in PAH-DNA binding when the dose applied is raised fro, 0.1 to 1.0 µmoles (table D.2.5). Using these factors it is possible to estimate the levels of DMBA and B[a]P-DNA binding in Swiss mice at the 1.0 µmole dose level. The estimated levels of DMBA and B[a]P-PAH binding are 30.0 and 16.6 pmoles adduct/mg DNA respectively.

From this data it is possible to estimate the PAH-DNA binding order in Swiss mice at the 1.0 µmole dose level.

DMBA (30 pmoles/mg DNA) > 3-MC (24.5 pmoles/mg DNA) > B[a]P (16.6 pmoles/mg DNA)

This agrees with the order suggested by Slaga et al. (JSLA82). Slaga, however, reports a 2 fold difference in binding potency between 3-MC and B[a]P. The data presented here show a 3-MC:B[a]P binding ratio of 1.5. It should be noted that the DNA binding ranking proposed by Slaga et al. is based on *in vitro* data (JSLA82). Therefore, it may not adequately reflect the *in vivo* binding behaviour of these compounds. It is also important to recognize that the PAH-DNA binding values for both DMBA and B[a]P are estimated from binding patterns within a single mouse strain. Further, in using these factors, a linear relationship between dose and binding is assumed. The binding dose-response curves for DMBA and B[a]P shown in figure D.4.1-3 indicated that the relationship is not linear. In addition, the curves suggest that as the dose increases, the rate at which binding increases begin to decline. These factors lower the confidence in the estimated values for both DMBA and B[a]P. As a result, confidence in the estimated binding order is lowered. Additional data on DMBA, B[a]P and 3-MC binding at fixed doses would aid in establishing the validity of the order suggested above. Ranking DMBA higher than both 3-MC and B[a]P is not likely to be incorrect. However, as will be seen in later discussions, there is some controversy as to the proper ranking of B[a]P and 3-MC in other mouse strains.

D.4.1.1.2.1.3 PAH-DNA Total Binding in C57BL Mice

In C57BL mice, the PAH-DNA binding order differs from that observed in Swiss mice. At the 1.0 µmole dose level, the observed order is (table D.4.1-5) as follows.

DMBA (43pmoles/mg DNA) > B[a]P (27 pmoles/mg DNA) ≥ 3-MC (24 pmoles/ mg DNA)

Because the binding data is available at a dose common to all three compounds, there is no need to estimate binding levels. Therefore the confidence in the suggested order is greater than that in the order proposed for Swiss mice. As can be seen in table D.4.1-5 the difference between B[a]P and 3-MC binding levels is slight. Therefore, the two compounds could reasonably be considered to be equal.

Table D.4.1-5: PAH-DNA Total Binding in C57BL Mice 19 Hours After Topical Application of PAH

Compound	Dose (µmoles)	Total Binding (pmoles/mg DNA)	Reference
B[a]P	0.1	4.2	JPHI78
B[a]P	1.0	27.0	JPHI79
DMBA	0.1	10.4	JPHI78
DMBA	1.0	43.0	JPHI79
3-MC	1.0	24.0	*

* Value averaged from JPHI78, JPHI79 (table D.4.1-3)

D.4.1.1.2.1.4 PAH-DNA Total Binding in DBA/2 Mice

The available PAH-DNA binding data for DMBA, B[a]P and 3-MC in DBA/2 mice is summarized in table D.4.1-6. For DBA/2 mice , as with Swiss mice, a direct comparison between all three compounds is not possible. Applying the previously derived correction factors for DMBA (4) and B[a]P (6.5) it is possible to estimate the total binding at the 1.0 µmole dose level. The cautions which applied to the estimated values for Swiss mice must also be applied to the data for DBA/2 mice. When levels are calculated in the manner outlined above (Section D.4.1.2.1.2) a PAH-DNA binding order for DBA/2 mice can be estimated as follows.

DMBA (46 pmoles/mg DNA) > B[a]P (39 pmoles/mg DNA) > 3-MC (24 pmoles/mg DNA)

Table D.4.1-6: PAH-DNA Total Binding in C57BL Mice 19 Hours After Topical Application of PAH

Compound	Dose (µmoles)	Total Binding (pmoles/mg DNA)	Reference
B[a]P	0.1	5.9	JPHI78
	1.0	39	Estimated
DMBA	0.1	11.0	JPHI78
	1.0	46	estimated
3-MC	1.0	23.8	JPHI79

D.4.1.1.2.1.5 PAH-DNA Total Binding: Individual Strains; Conclusions

The data in tables D.4.1-3 through D.4.1-6 show that of the three PAH for which adequate data is available, DMBA has the highest PAH-DNA binding affinity in all strains of mice tested. This agrees with the relative binding and tumorigenic potencies reported by various authors (JSLA82, JPHI79). Although it is clear that both B[a]P and 3-MC are less efficient at binding to DNA than DMBA, it is not clear what the relative ranking of these two compounds should be. In Swiss mice, the apparent ranking is DMBA > 3-MC > B[a]P. For both C57BL and DBA/2 mice the data indicate that B[a]P has a higher PAH-DNA binding potential than 3-MC. In C57BL mice, the difference between B[a]P and 3-MC-DNA binding is almost negligible. The two values could be considered equal within the limits of experimental data. Thus, the binding order in C57BL would be; DMBA > B[a]P ≥ 3-MC. In DBA/2 mice the levels of PAH-DNA binding for B[a]P and 3-MC are sufficiently dissimilar (3-MC:B[a]P binding ratio = 0.62 Section D.4.1.2.1.4) to rank the PAH as follows; DMBA > B[a]P ~ 3-MC.

It should be noted that the C57BL is the only mouse strain for which a direct comparison of PAH-DNA binding levels for all three compounds can be made. Therefore, the level of confidence in the this ranking must be higher than that in either the Swiss or DBA/2 strains. The rankings for these latter two strains are based on estimated binding levels for two of the three compounds in each group. Therefore, the final order of PAH-DNA binding would be the following.

DMBA > B[a]P = 3-MC

A clearer resolution of the relative rankings of B[a]P and 3-MC is not possible within the limits of the currently available data.

D.4.1.1.2.2 PAH-DNA Total Binding: Inter-Strain Comparisons

The data presented in the previous sections indicates that, within individual mouse strains, the PAH-DNA binding affinities of DMBA, B[a]P and 3-MC correlate well with the reported tumorigenic

potential of these compounds (JDIG84, JPHI79). Further, the correlation appears to hold for all strains examined. In SENCAR, Swiss, C57BL and DBA/2 mouse strains, DMBA shows the highest level of binding and tumorigenic response. Benzo[a]pyrene and 3-MC show lower levels of binding and correspondingly lower tumorigenic responses (see Section D.4.1.2.1 and JDIG84, JPHI79).

A number of authors have examined the PAH-DNA binding of individual PAH across a number of mouse strains to determine if strain differences in tumorigenic response correlates with strain differences in the levels of PAH-DNA adduction (JPHI78, JPHI79, JDIG84, JSLA82, JBUR83). Such a correlation would require that the levels of PAH-DNA binding for a specific PAH would decrease across strains as tumorigenic response decreased. Phillips et al. (JPHI78, JPHI79) and DiGiovanni et al. (JDIG84) looked at PAH-DNA binding in Swiss, C57BL and DBA/2 mouse strains. Both groups found that the levels of binding were essentially the same in the responsive (Swiss), neutral (C57BL) and non-responsive (DBA/2) mouse strains. From this the authors concluded that inter-strain correlations between PAH-DNA binding and tumorigenic response either do not exist or are extremely poor. The following sections examine the available PAH-DNA binding data for DMBA, B[a]P and 3-MC in SENCAR, Swiss, C57BL and DBA/2 mice to determine the validity of the conclusions presented by Phillips et al. (JPHI78, JPHI79) and DiGiovanni et al. (JDIG84).

D.4.1.1.2.2.1 Inter-Strain Comparisons for DMBA-DNA Binding

The formation of DMBA-DNA adducts in Swiss, C57BL and DBA/2 mice 19 hours post treatment is summarized in table D.4.1-7. There is little difference in the levels of adduct formation between the C57BL and DBA/2 mouse strains (table D.4.1-7). The level of DMBA-DNA binding in Swiss mice is only 68 percent of the level of the other two strains. All three strains were tested in the same experiment which suggests that the differences in binding are real and not due to inter-experiment variation. Based on the data the ranking of the three strains with respect to DMBA-DNA binding is the following.

DBA/2 = C57BL > Swiss

Table D.4.1-7: DMBA-DNA Binding in Mouse Epidermal DNA; 19 Hours Post Treatment

Dose (μmoles)	Strain (Sex)	Total Binding (pmoles adduct/mg DNA)	Reference
0.10	Swiss (M)	72.	JPHI78
	C57BL (M)	10.4	JPHI78
	DBA/2 (M)	10.6	JPHI78

The formation of DMBA-DNA adducts in SENCAR, C57BL and NIH Swiss mice 24 hours post treatment is summarized in table D.4.1-8. From the data in table D.4.1-8 it is clear that DMBA-DNA binding in SENCAR mice greatly exceeds PAH-DNA binding in the other strains tested. At both doses 0.01 and 0.10 μmoles, there is a 2.5 to 3.0 fold difference in the levels of adduct formation. It should be noted however, that the PAH-DNA binding was measured in female SENCAR mice and in male C57BL and NIH Swiss mice. Bigger et al. looked at DMBA-PAH adduct formation in epidermal tissue from male and female NIH Swiss mice (JBIG83). while the adduct profiles in both sexes were essentially the same, the total adduct formation in male epidermal DNA was 1.5 fold higher than that reported in the female (JBIG83). This suggests that the difference in binding between SENCAR and C57BL and NIH Swiss mice of the same sex would be greater than the 2.5 to 3.0 fold reported here.

From the data it can be seen that DMBA-DNA binding in SENCAR mice is greater than either C57BL and NIH Swiss. Ranking the remaining two strains is perhaps less clear. At both doses tested the levels of DMBA-DNA binding are 70 to 80 percent lower in the NIH Swiss strain than in the C57BL strain. Therefore the overall order for the three mouse strains with respect to DMBA-DNA binding would be the following.

SENCAR > C57BL > NIH Swiss

Table D.4.1-8: DMBA-DNA Binding in Mouse Epidermal DNA; 24 Hours Post Treatment

Dose (μmoles)	Strain (Sex)	Total Binding (pmoles adduct/mg DNA)	Reference
0.01	SENCAR (F)	3.8	*
	C57BL (M)	1.7	JDIP84
	NIH Swiss (M)	1.2	JDIP84
0.10	SENCAR (F)	16.5	JDIG86A
	C57BL (M)	5.5	JDIP84
	NIH Swiss (M)	4.6	JDIP84

* Average of JDIG83 (3.6 pmoles/mg DNA), JDIG86 (3.9) and JDIG86A (3.9)

D.4.1.1.2.2.2 Inter-strain Comparisons for B[a]P-DNA Binding

The formation of B[a]P-DNA adducts in Swiss, C57BL and DBA/2 mice 19 hours post treatment is summarized in table D.4.1-9. The levels of total binding in C57BL and Swiss mice are 70 percent and 45 percent of the levels reported for DBA/2 mice (JPHI78). In each case, the data are averages of two experiments (JPHI78). The lack of sufficient data for adequate statistical comparisons of the strain differences means that ranking these strains must be done with caution. Based on the data in table D.4.1-9 the ranking of the three strains with respect to B[a]P-DNA binding is the following.

DBA/2 > C57BL > Swiss

Table D.4.1-9: B[a]P-DNA Binding in Mouse Epidermal DNA; 19 Hours Post Treatment

Dose (μmoles)	Strain (Sex)	Total Binding (pmoles adduct/mg DNA)	Reference
0.10	Swiss (M)	2.6	JPHI78
	C57BL (M)	4.2	JPHI78
	DBA/2 (M)	5.9	JPHI78

The formation of B[a]P-DNA adducts in SENCAR and C57BL mice 24 hours post treatment is summarized in table D.4.1-10. The levels of B[a]P-DNA binding in the SENCAR strain are 3.6 fold higher than those found in the C57BL strain. It should again be noted that the

binding data are in two sexes. Therefore the real difference in total B[a]P-DNA binding between the two strains of the same sex would be expected to be greater than the difference noted here (See Section D.4.1.2.2.1 for discussion) (JBIG83).

Table D.4.1-10: B[a]P-DNA Binding in Mouse Epidermal DNA; 24 Hours Post Treatment

Dose (μmoles)	Strain (Sex)	Total Binding (pmoles adduct/mg DNA)	Reference
0.20	SENCAR (F)	12.4	*
	C57BL (M)	3.4	JASH82

* average of JDIG85 (9.6 pmoles/mg DNA), JDIG86 (12.6) and JSMO87 (15.0)

Based on the data in table D.4.1-10, the ranking of the two strains with respect to B[a]P-DNA binding is as follows.

SENCAR > C57BL

D.4.1.1.2.2.3 Inter-Strain Comparisons for 3-MC-DNA Binding

The formation of 3-MC-DNA adducts in Swiss, C57BL and DBA/2 mice 19 hours post treatment is summarized in table D.4.1-11. The variation in adduct formation is sufficiently small (range 23.8 to 24.5) so that the levels of adduct formation in all three strains can be considered equal. Therefore, the ranking of all three strains with respect to 3-MC-DNA binding would be as follows.

Swiss = C57BL = DBA/2

Table D.4.1-11: 3-MC-DNA Binding in Mouse Epidermal DNA; 19 Hours Post Treatment

Dose (μmoles)	Strain (Sex)	Total Binding (pmoles adduct/mg DNA)	Reference
1.0	Swiss (M)	24.5	JPHI78
	C57BL (M)	24.0	*
	DBA/2 (M)	23.8	JPHI78

* average of JPHI78 (23.0 pmoles/mg DNA), JPHI79 (25.0)

D.4.1.1.2.2.4 Inter-Strain Comparisons; Conclusions

DiGiovanni et al. have ranked a number of mouse strains based on their reported sensitivity to both complete and two-stage carcinogenesis assays (JDIG84). In the complete carcinogenesis assay the strains were ranked as follows.

SENCAR > CD-1 > C57BL ≥ BALB/c ≥ ICR/HA Swiss > CH3 > DBA/2

In the two-stage carcinogenesis assay the strains were ranked as:

SENCAR > CD-1 > ICR/HA Swiss > BALB/c > C57BL > CH3 > DBA/2

The experimental protocols used for PAH-DNA total binding data more closely resemble the complete carcinogenesis assay than the two-stage assay as a promoter is not used in the PAH-DNA total binding experiments. Therefore, an abbreviated version of the complete carcinogenesis assay ranking proposed by DiGiovanni et al. (JDIG84) will be used as the basis for comparison between tumorigenic sensitivity and PAH-DNA total binding. The abbreviated ranking used in this discussions is:

SENCAR > C57BL ≥ ICR HA Swiss > DBA/2

The ICR/HA Swiss strain is used in place of the Swiss and NIH Swiss strains for which PAH-DNA total binding data is available.

The PAH-DNA total binding presented in sections D.4.1.2.2.1 and D.4.1.2.2.2 clearly indicate that PAH-DNA binding in SENCAR mice is greater than in other strains. The binding of DMBA in SENCAR mice is 2.5 to 3.0 fold greater than that reported for either C57BL or NIH Swiss mice (see table D.4.1-8). The binding of B[a]P in SENCAR mice is a minimum of 3,6 fold greater than that reported for C57BL mice (see table D.4.1-10). This data would seem to confirm the ranking of SENCAR mice as greater than other strains with respect to PAH-DNA binding.

The PAH-DNA total binding data at 19 hours post treatment presented in sections D.4.1.2.2.1 and D.4.1.2.2.2 demonstrate that the ranking of strains other than SENCAR is less clear. The PAH-DNA total binding data for DMBA (see table D.4.1-7) suggests that PAH-DNA binding in DBA/2 and C57BL mice is approximately equal and that binding in both is marginally greater than that noted in Swiss mice. The equivalent data for B[a]P-DNA binding (see table D.4.1-9) indicates that total binding in the DBA/2 mouse strain is greater than that of either the C57BL or Swiss strains. Although the differences in B[a]P-DNA total binding between DBA/2 C57BL and Swiss mice cannot be considered as negligible, they must be considered as minimal when compared to the difference between all three strains and the SENCAR mouse strain. The 3-MC-DNA total binding data presented in table D.4.1-11 shows that the DBA/2, C57BL and Swiss mouse strains all bind 3-MC with equal avidity. Therefore based on these data, the ranking for these three strains would be the following.

Swiss = C57BL = DBA/2

In conclusion, the difference in PAH-DNA total binding between SENCAR mice and the other strains, and the lack of a definitive pattern within the other strains suggests a final ranking of the four strains with respect to PAH-DNA total binding as follows.

SENCAR > Swiss = C57BL = DBA/2

Thus, it can be stated that a moderate inter-strain correlation between tumorigenic response and PAH-DNA binding does exist. The SENCAR mouse strain, which is reported to be the most sensitive to the tumorigenic effects of PAH (JDIG84) also shows the highest level of PAH-DNA binding. Based on the PAH-DNA binding data presented herein, correlations between tumorigenic response and PAH-DNA binding in epidermal tissue for the remaining strains (Swiss, C57BL and DBA/2) does not appear to exist.

D.4.1.1.3 PAH-DNA Total Binding: Inter-Species Comparisons

The majority of available *in vivo* PAH-DNA total binding data exists for PAH binding in mouse epidermal DNA. Two reports in the literature have compared the formation of B[a]P-DNA adducts in epidermal tissue of mice and rats following topical application of B[a]P (JBAE81, JROJ86). The data are summarized in table D.4.1-12. In both studies, the level of adduct formation was lower in the rat than in the mouse.

Accurate comparisons of adduct levels between species requires that the skin surface ares over which the dose is applied be known. This insures that dosages and adduct levels may be compared on a per unit area basis. For example, a compound administered to both mice and rats is likely to be distributed over a larger skin surface area in the rat than in the mouse. Consequently, the dose per unit area will be lower. Therefore, a lower level of adduct formation in rats may be the result of one of the following factors.

i) a lower level of compound available per unit area or;

ii) a true lower level of adduct formation or;

iii) a combination of these two factors.

Failure to consider factors of this nature may lead to erroneous conclusions regarding differences in the levels of PAH-DNA binding between species.

Table D.4.1-12: B[a]P-DNA Total Binding in Epidermal Tissue of Mice and Rats After Topical Apllication of B[a]P

Species (Strain)	Skin Area (cm^2)	Dose (μmoles)	Dose/cm^2	Total Binding*	Total/cm^2	Ref
Mouse (Swiss F)	9	0.25	0.028	33.3	3.7	JBAE81
Rat (Wistar M)	36	0.50	0.014	9.9	0.28	JBAE81
Mouse (Swiss M)	?	0.25		7.1	?	JROJ86
Rat (Wistar M)	?	0.75		6.9	?	JROJ86

* Total Binding is pmoles adduct/mg DNA

The work of Baer-Dubowska et al. (JBAE81), provides the treated skin areas for both rats and mice (Table D.4.1-12). Therefore it is possible to calculate both dose and total binding on a unit area basis. This does however assume that the applied dose is distributed evenly over the entire treatment area. The dose per unit area and total binding per unit area are provided in table D.4.1-12. The data show a 13.5 fold difference in the B[a]P-DNA total binding per unit area between mice and rats. However, the mice were treated with a dose per unit area two fold greater than that applied to rat skin. Therefore, if both species were treated with the same dose per unit area the predicted difference in the level of adduct formation would be 6.8 fold. This assumes that either, doubling the dose in the rat from 0.014 μmoles/cm^2 to 0.028 μmoles/cm^2 would double the level of adduct formation or lowering the dose given to mice from 0.028 μmoles/cm^2 to 0.014 μmoles/cm^2 would result in a 50 percent reduction in the level of adduct formation. The lack of adequate dose-response data for either species make accurate predictions of the binding behaviour in either species impossible.

The work of Rojas et al. does not specify the treatment surface area in either species (JROJ86). Therefore, it is difficult to evaluate the data in a quantitative manner. Qualitatively, B[a]P-DNA binding in the rat appears to be approximately 3 fold lower than that observed in the mouse (JROJ86) (table D.4.1-12). Without knowing the surface areas over which the dosages were applied in either species it is difficult to make quantitative estimates of differences in species sensitivity.

Both sets of data presented in table D.4.1-12 would suggest that the formation of B[a]P-DNA adducts in lower in the rat than in the mouse. This would agree with the reported lower sensitivity of the rat to the tumorigenic potential of PAH (JBAE81). Unfortunately, there is insufficient data to make quantitative estimates of the magnitude of the difference in PAH-DNA total binding between mice and rats with any degree of confidence. Total binding data can be used as a qualitative explanation for the observed difference in species sensitivities.

D.4.1.2 PAH-DNA Total Binding Following Oral Administration of PAH

There is little adequate data in the literature regarding PAH-DNA total binding following oral administration of PAH. Two studies have examined total adduct formation in a variety of tissues from two strains of mice following oral administration of B[a]P (JLU*86, JSTO84). The data are summarized in table D.4.1-13.

Adduct formation was monitored at different times in the two studies cited in table D.4.1-13. Therefore, it is difficult to identify similarities or differences in adduct formation between the two strains. In ICR mice, at 24 hours, the highest adduct levels are found in the liver followed by lung, kidney and brain (JLU*86). In A/HeJ mice, at 48 hours, the highest adduct levels are found in the lung followed by liver, brain and kidney (JSTO84). This apparent change in order may be due to strain differences. However, it is more likely to reflect the normal pattern of adduct loss from the various tissues. If the rate of adduct loss is greater in the liver than in other tissues, the change in PAH-DNA adduct levels in the liver between 24 and 48 hours may be greater than that in lung. This change may be of sufficient magnitude to result in the changes seen in table D.4.1-13. In the absence of time-course studies which monitor adduct loss, or studies which determine PAH-DNA adducts levels in multiple strains at a single time point, it is difficult to determine the significance of the observed differences.

The lack of data for compounds other than B[a]P coupled with the limited validity of the available data makes it impossible to draw any meaningful conclusions regarding similarities or differences in PAH-DNA adduct formation between strains following oral administration of PAH.

Table D.4.1-13: B[a]P-DNA Total Binding in Female ICR and A/HeJ mice Following Oral Administration of B[a]P

Strain (Sex)	Dose (μmoles)	Time (hours)	Total Binding (pmoles adduct/mg DNA)				Ref.
			Lung	Liver	Kidney	Brain	
ICR (F)	10	24	1.3	1.5	0.73	0.36	JLU*86
A/HeJ (F)	2.4	48	0.04	0.022	0.017	0.016	JSTO84
A/HeJ (F)	24	48	8.3	7.3	4.3	4.5	JSTO84

D.4.1.3 PAH-DNA Total Binding Following Inhalation Administration of PAH

Data for this section appears to be limited to one study in rats (JWEY87). Weyand et al. administered 0.025 μmoles of B[a]P to male Sprague-Dawley rats via intratracheal installation. Adduct levels were monitored in lung and liver 6 hours post treatment (Table D.4.1-14). Although the levels of adduct formation in both tissues were low, those in the liver were found to be 2.5 fold greater than those in the lung. No other information on total adduct formation following any form of inhalation administration was found.

Table D.4.1-14: B[a]P-DNA Total Binding in Male Sprague-Dawley Rats 6 hours Post Intratracheal Installation

Dose (μmoles)	Total Binding (pmoles adduct/mg DNA)		Ref
	Lung	Liver	
0.025	0.013	0.033	JWEY87

D.4.1.4 PAH-DNA Total Binding: Summary of Conclusions

The available literature on PAH-DNA total binding has been examined for the dermal, oral and inhalation routes of exposure. Each of these routes is summarized below. The majority of the available information deals with adduct formation in mouse epidermal tissue following topical treatment. Data for the remaining routes is sparse or nonexistent.

D.4.1.4.1 Topical Administration; Summary of Conclusions

There exists a moderate amount of data on PAH-DNA total binding in mice following topical administration of a number of PAH including DMBA, B[a]P and 3-MC (see tables D.4.1-3 through D.4.1-12). The data show a good correlation between PAH-DNA total binding and tumorigenic potency within a single mouse strain (JSLA82,JDIG84,JPHI78,JPHI79,JBUR83). Within a given mouse strain the order of PAH-DNA binding is as follows.

DMBA > B[a]P = 3-MC

The good correlation within a single strain suggests that PAH-DNA total binding may be useful in predicting tumorigenic response within individual mouse strains.

Inter-strain comparisons of PAH-DNA total binding and tumorigenic potency show that SENCAR mice exhibit the highest level of PAH-DNA binding (Section D.4.1.1.2.2) and are the most sensitive to the tumorigenic effects of PAH (JDIG84). However, correlations between PAH-DNA binding and tumorigenic potency do not hold for the other three strain examined (Swiss, C57BL and DBA/2). The levels of PAH-DNA binding in the responsive Swiss and C57BL mouse strains do not differ substantially form those in the non-responsive DBA/2 mouse strain (Section D.4.1.1.2.2) (JPHI78,JPHI79). Thus a moderate inter-strain correlation between PAH-DNA binding and tumorigenic potency can be said to exist. The ranking that arises from this correlation is as follows.

SENCAR > Swiss = C57BL = DBA/2

A number of reasons have been suggested to explain the lack of a reasonable correlation between PAH-DNA total binding and tumorigenic potency across mouse strains. They include the following.

i) differences in strain response to tumor promotion with TPA. This is discussed in some detail by DiGiovanni et al. (JDIG84). The basic premiss is that; binding is essentially the same in all mouse strains. It is the sensitivity of each strain to promotion with TPA that determines the tumorigenic response. The data presented here, however, show that PAH-DNA binding in SENCAR mice is greater than in the other strains tested. It is possible that response to TPA promotion may be an important factor in determining tumorigenic in strains other than SENCAR. There is insufficient data available to evaluate this suggestion.

ii) PAH-DNA total binding fails to look at the levels of specific PAH-DNA adducts. It is possible that the difference in strain response results, in part, from differences in the levels of formation of critical PAH-DNA adducts (JPHI78, JPHI79). This will be examined in subsequent section of this report.

iii) Total Adduct formation studies fail to examine adduct persistence. Nor do they monitor changes in adduct profile with time. Time-course studies that examine the loss of total adducts cannot account for changes in adduct profiles as a function of time. It is possible that alterations in adduct profiles between strains may, in part, be responsible for the observes strain differences in tumor sensitivity (JPHI78, JPHI79). This will be discussed in later sections of this report.

D.4.1.4.2 Oral Administration; Summary of Conclusions

There is insufficient data to draw any meaningful conclusions from this section.

D.4.1.4.3 Inhalation Administration; Summary of Conclusions

There is insufficient data to draw any meaningful conclusions from this section.

D.4.1.5 PAH-DNA Total Binding: Summary of Missing Data

There is so much missing data it is difficult to know where to begin. There is some data for dermal exposure (topical application of PAH) but data on further PAH is needed. In addition, data in more strains at the same time point would strengthen the conclusions drawn from this section. Inter-species comparisons are difficult if not impossible as there is data for 2 rat studies and nothing else. Data of the appropriate kind in more than one species would be useful. It would be better if this inter-species data came from the same experiment as this would make comparisons stronger.

There is no adequate data for routes other than dermal application. This makes it difficult to use this section for anything useful. Data following oral administration in a number of tissues for a number of strains and species is needed to strengthen correlations in this area.

D.4.2 PAH-DNA Adduct Profiles

In general, two types of DNA adducts can be formed, covalently bound and non-covalently bound (BGEA88, JJAN90, JLU*91). The non-covalently bound adducts are formed by an intercalation of the PAH metabolites between the stacked base pairs within the DNA core. This type of complex is usually formed at sites on the DNA where hydrolysis of an incoming diol-epoxide metabolite favours the formation of a tetrol rather than a covalently bound complex (BGEA88). Despite the lack of covalent bonds, these types of adducts can be surprisingly stable if there is adequate hydrogen bonding between the adduct and the bases within the DNA. Covalent DNA adducts are created by the formation of a chemical bond between the PAH metabolite and one of the DNA bases (BGEA88).

The formation of covalent bonds requires that the metabolite contains an electrophilic center that can undergo nucleophilic substitution during the alkylation process. There are a number of PAH metabolite structures that meet this requirement (BGEA88, JJAN90, JLU*91, BKAK78, BBAI88, BWEI78, JROG83, JBAE90, JCHA89, JBOR81, JDIP90, JMAH86). These are listed in table D.4.2-1. Epoxides are the most common structural feature that provides this requirement. Although most epoxides are, in theory, able to undergo these types of reactions, in practice, only those compounds where the epoxide is sufficiently stable appear capable of forming these types of modification. This is usually restricted to epoxides formed in the K-regions of some PAH, but more typically, to epoxides formed in the bay region in conjunction with a diol on the remaining two carbons of the benzylic ring (vicinal diol-epoxide). Other reactive metabolites are also capable of forming bonds with cellular macromolecules. These include some radicals formed as a result of one electron oxidations of the PAH parent. The oxidations can be carried out by either cytochrome P-450 or by prostaglandin synthetase. Adducts derived from PAH quinone and phenol metabolites have been identified in vivo (JBOR81), however, the adduct complexes have not been characterized.

Table D.4.2-1: Reactive PAH Metabolites

Reactive Metabolite	Modification Site	Reference
Primary Epoxides	Guanine	JBAE90, JCHA89, JBOR81
Diol-Epoxides	Guanine Adenine Cytosine Phosphodiester	JLU*91, JDIP90 JLU*91, JDIP90 JDIP90, JMAH86 BKAK78
Quinones	Unidentified	JBOR81
Phenols	Unidentified	JBOR81
PAH Radicals	Guanine	JLU*91, JROG83

The primary site of alkylation is the exocyclic amino groups of the guanine, adenine and cytosine. Thymidine which does not have an exocyclic amino group does not appear to be a site for DNA modification by PAH metabolites (BGEA88,BKAK78,BBAI88). There are reports of covalent adducts being formed between the PAH metabolites and the purine or pyrimidine core, but these adducts are not common (JLU*91,BBAI88). The DNA bases and the locations of adduct formations are listed in table D.4.2-2.

Table D.4.2-2: DNA Adducts; Bases and Sites of Formation

DNA Base	Adduct Location	Reference
Guanine	N2 N7 C8	BBAI88, JROJ86 JLU*91, BBAI88 JCHA89
Adenine	N6	BBAI88
Cytosine	N4	BBAI88, JMAH86

The formation of DNA adducts with PAH diol-epoxides is a multistage process (JJER91). The initial step is a rapid intercalation of the diol-epoxide between the stacked DNA bases. This complexation is followed by a slower hydrolysis reaction that opens the epoxide ring to form either a PAH tetrol or a DNA-PAH adduct. These adducts are tetrol derivatives in which the exocyclic amino group of the DNA base replaces the hydroxyl group at the benzylic carbon of the epoxide (JJER91). For most diol-epoxides studied to date, tetrol formation appears to be the predominant reaction. Adduct formation, which can be viewed as a competing reaction, accounts for between two to 30 percent of the total (JJER91).

Two types of DNA binding sites have been identified; Type "A" and Type "B", (BGEA88,JJAN90,JLU*91,JJER91). Binding to type "A" sites causes a reorientation of the molecule from an intercalated location to an exterior position in either the major or minor groove of the DNA double helix (BGEA88,JJAN90,JLU*91,JJER91). Complexation to guanine produces adducts that lie in the minor groove while binding to adenine produces adducts that lie in the major groove (JLU*91). The degree of intercalation between the bases is markedly reduced for these adducts (JJAN90,JJER91). A number of diol-epoxides give rise to this type of adduct, including; (+) BPDE-II (63323-31-9), Cholanthrene, 9,10-diol-7,8-epoxide-3-methyl-, and Chrysene-1,2-diol-3,4-epoxide-5-methyl-, (BGEA88,JJER91). At type "B" sites, covalent binding does not cause a reorientation of the molecule. The planar core of the diol-epoxide lies nearly parallel with the plane of the DNA bases (JJAN90). In general, compounds that form type "A" adducts tend to be more biologically active than those that form type "B" adducts (BGEA88,JJAN90,JLU*91,JJER91).

The formation of DNA adducts has been investigated for a number of PAH including Benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, benz[a]anthracene, chrysene, dibenz[a,j]anthracene, benzo[b]triphenylene, benzo[c]phenanthrene and 3-methylcholanthrene (see Table D.4.2-3 and Table D.4.2-4). Of these compounds, B[a]P has been the most intensively investigated. The following sections will focus on the specific adducts derived from B[a]P.

D.4.2.1 Formation of Specific B[a]P-DNA Adducts

The binding of B[a]P metabolites to DNA has been extensively studied both *in vitro* and *in vivo* in a number of different species (BKAK78, BBAI88, BWEI78, JROG83, JBAE90, JCHA89, JBOR81, JDIP90, JMAH86, JROJ86, JDIG85, JBAE81, JASH82, JRED90, JGIN90, JMEL90, JSTO84). Results indicate that a number of B[a]P metabolites including phenols, quinones, epoxides, diol-epoxides and radicals are sufficiently reactive to form covalent bonds with DNA. A list of reactive B[a]P metabolites and the DNA binding locations is provided in Table D.4.2-4. Although there is evidence for the formation of B[a]P phenols and quinone adducts, the DNA binding sites and the structures of these complexes is, as yet, unknown (JBOR81).

There is limited information available regarding the binding of B[a]P-epoxide metabolites. The K-region epoxide of B[a]P ((±)-B[a]P-4,5E) is the only epoxide for which information is available, the binding of this compound to DNA is directed toward the exocyclic amino group of guanosine. It is unclear if the covalent bond is formed between C4 or C5 of the epoxide (JBAE90,JBOR81,JDIG85). Two of the three studies that report the formation of this adduct have identified it as a 9-hydroxy derivative of (±)-B[a]P-4,5E (JBAE90,JDIG85). It was suggested that this compound arose from an initial hydroxylation of B[a]P at C9 followed by the formation of the K-region epoxide and C4 and C5 (JBAE90,JDIG85). The remaining report did not fully characterize the B[a]P-metabolite responsible for the B[a]P-epoxide like adduct (JBOR81). It is possible that the same B[a]P-9OH-4,5-E product was involved. This would not be inconsistent with reported PAH metabolism pathways (see Section D.1).

The formation of BPDE-DNA adducts has received the greatest amount of scientific attention because it is believed that the formation of these adducts is one of the critical events in the initiation of mutagenesis and tumorigenesis (BGEA88). The formation of a BPDE-DNA bond occurs between C10 of BPDE and the exocyclic amino group of the purine and pyrimidine bases (BBAI88). Studies have shown that BPDE can form adducts with any nucleic acid the contains an exocyclic amino group including; RNA *poly*G, *poly*A, *poly*C, *poly*dG, *poly*dA and *poly*dC (BBAI88). In addition there is some evidence that BPDE is able to bind to the phosphodiester backbone of DNA resulting in strand breakage (BKAK78,BBAI88,BWEI78). This result is, so far, confined to tests with single strand DNA. There is no evidence that this binding occurs in native DNA (BBAI88). Histone and non-histone protein are also sites of BPDE binding. Binding to proteins may be governed by the three dimensional conformation of the protein (BWHI78). Although BPDE is capable of binding to protein there appears to be a preferential binding to DNA. This may be due to the greater number of nucleophilic acceptor groups on DNA compared to protein (BWHI78).

The binding of (±)-BPDE-II to the exocyclic amino group of dG proceeds through a *trans* opening of the epoxide ring at C10. The amino group approaches the benzylic carbon from the opposite face of the epoxide group (BBAI88). A small amount of *cis* opening product has also been identified (BBAI88). The binding of (±)-BPDE-II and (+)-BPDE-II to dA produces both *cis* and *trans* addition products (BBAI88). The binding of (±)-BPDE-I to dG also produces both *cis* and *trans* products (BBAI88).

The binding of BPDE to dG leaves two of the three G=C hydrogen bonds intact producing a relatively stable PAH-DNA complex. Binding to dA disrupts the hydrogen bonding of the A=T pair causing localized denaturation of the DNA double helix (BKAK78). It has been suggested that this binding to dA and the subsequent denaturation is a more important factor in the mutagenic and tumorigenic activities of this compound than the formation of dG adducts (BBAI88). There is limited evidence to support this (BBAI88, JCHA89, JJER91).

All of the B[a]P-DNA adducts listed in table D.4.2-4, with the exception of those bound to dC have been identified *in vivo*. Adducts derived from BPDE are the predominant one reported and have been found in all species and strains studied (see table D.4.2-5). Adducts derived from B[a]P-4,5-E have been reported in mice (JBAE81,JROJ86,JDIG85,JBAE81,JASH82,JRED90,JGIN90) and rats (JROJ86,JBAE81), but not in hamsters (JMAEL90) or rabbits (JSTO84). It is difficult to determine if B[a]P-4,5E adducts were not formed in these latter species or if the studies failed to identify them. These studies were looking for adducts other than those derived from BPDE (JMEL90,JSTO84). Adducts derived from phenols and quinones have only been identified in rats (JBOR81). However, because the DNA bases involved in the formation of these adducts, they have not been included in table D.4.2-4. In many of the *in vivo* studies, there are a number of adducts which have been identified but not characterized (JBOR81, JDIG85, JBAE81, JASH82, JRED90, JGIN90, JSTO84, JADR83, JLUL84). While there is only limited evidence to support it, it would not be unreasonable to expect that these adducts are derived from phenol, quinone and radical B[a]P metabolites.

Table D.4.2-3: List of Abbreviations for PAH Metabolites and PAH-DNA Adducts.

Abbreviation	Compound	CAS #	ABS
(±)-BPDE-I	Benzo[a]pyrene, 7α,8ß-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (±)	58917-91-2	
(+)-BPDE-I	Benzo[a]pyrene, 7α,8ß-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+)	63323-29-5	7S,8R,9S,10R
(-)-BPDE-I	Benzo[a]pyrene, 7ß,8α-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (-)	63357-09-5	7R,8S,9R,10S
(±)-BPDE-II	Benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (±)	58917-67-2	
(+)-BPDE-II	Benzo[a]pyrene, 7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+)	63323-31-9	7R,8S,9S,10R
(-)-BPDE-II	Benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (-)	62232-30-8	7S,8R,9R,10S
6MeB[a]P	Benzo[a]pyrene, 6-methyl-,	2381-39-7	
(±)-B[a]P-4,5E	Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-, (±)	64437-52-1	
B[a]P-9OH-4,5E	Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-9-hydroxy-, (±)		
DMBA	Benz[a]anthracene, 7,12-dimethyl-,	57-97-6	
(±)-DMBA-DE-II	Benz[a]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)		
(±)-DMBA-DE-I	Benz[a]anthracene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)		
DMBA-5,6E	Benz[a]anthracene, 5,6-dihydro-5,6-epoxy-7,12-dimethyl-, (±)		
(±)-BAA-3,4D-1,2E	Benz[a]anthracene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±)	64598-81-8	
(±)-BAA-8,9D-10,11E	Benz[a]anthracene, 8α,9ß-dihydroxy-10ß,11ß-epoxy-8,9,10,11-tetrahydro-, (±)	64598-83-0	
(±)-Chry-1,2,9T-3,4E	Chrysene, 3α,4α-epoxy-1ß,2α,9-trihydroxy-1,2,3,4-tetrahydro-, (±)	88847-41-0	
( )DBAJA-3,4D-1,2E	Dibenz[a,j]anthracene, 3 ,4ß-dihydroxy-1 ,2 -epoxy-1,2,3,4-tetrahydro-, ( )		
( )-BBT-DE-I	Benz[b]triphenylene, 1 ,2ß-dihydroxy-3 ,4 epoxy-1,2,3,4-tetrahydro-, ( )		
( )-BBT-DE-II	Benzo[b]triphenylene, 1 ,2ß-dihydroxy-3ß,4ß-epoxy-1,2,3,4-tetrahydro-, ( )		
(-) BcP-DE-I	Benzo[c]phenanthrene, 3 ,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro- (-)	82510-59-6	
(+)-BcP-DE-I	Benzo[c]phenanthrene, 3ß,4 -dihydroxy-1 ,2 -epoxy-1,2,3,4-tetrahydro-, (+)	82510-56-3	
(+)-BcP-DE-II	Benzo[c]phenathrene, 3ß,4 -dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (+)	82510-58-5	
( )-3MC-DE	Cholanthrene, 9ß,10 -dihydroxy-7ß,8ß-epoxy-3-methyl-7,8,9,10-tetrahydro-, ( )		
dG	Deoxyguanosine		
dA	Deoxyadenosine		
dC	Deoxycytidine		
C10-N6-dA	Adduct formed between C10 on a PAH and the exocyclic amino group attached to C6 of Adenosine		

Table D.4.2-4: Identified PAH-DNA Adducts

Parent	Metabolite	Adduct	Reference
Benzo[a]pyrene	(+)-BPDE-II	C10-N2-dG	JBAE90, JROJ86, JDIG85 JBAE81, JASH82
		C10-N6-dA	JDIG85
		C10-N4-dC	BBAI88
	(-)-BPDE-II	C10-N2-dG	JROJ86, JBAE81, JDIG85
		C10-N6-dA	JDIG85
	(±)-BPDE-II	C10-N2-dG	JBAE90, JROJ86, JDIG85
	B[a]P-9OH-4,5E	C4/C5-N2-dG	JBAE90, JROJ86, JDIG85
	6MeB[a]P	C6-CH_2-N2-dG	JROG83
DMBA	(±)-DMBA-DE-II	C1-N2-dG	JBAE90, JSIN90
		C1-N6-dA	JBAE90, JSIN90
	(±)-DMBA-DE-I	C1-N6-dA	JBAE90, JSIN90
	DMBA-5,6E	C5/C6-N2-dG	JCHA89
Benz[a]anthracene	(±)-BAA-3,4D-1,2-E	C1-N2-dG	BBAI88
	(±)-BAA-8,9D-10,11E	C11-N2-dG	BBAI88
Chrysene	(±)-Chry-1,2D-3,4E	C4-N2-dG	BBAI88
	(±)-Chry-1,2,9-T-3,4E	C4-N2-dG	BBAI88
Dibenz[a,j]anthracene	(±)-DBAJA-3,4D-1,2E	C1-N2-dG	JCHA89
		C1-N6-dA	JCHA89
		C1-N4-dC	JCHA89
Benzo[b]triphenylene	(±)-BBT-DE-I	C3/C4-N2-dG	JJER91
		C3/C4-N6-dA	JJER91
	(±)-BBT-DE-II	C3/C4-N2-dG	JJER91
		C3/C4-N6-dA	JJER91
Benzo[c]phenanthrene	(-)-BcP-De-I	C1-N6-dA	JCHA89, JPRU87
		C1-N2-dG	JPRU87
	(+)-BcP-DE-I	C1-N6-dA	JCHA89
	(+)-BcP-DE-II	C1-N6-dA	JPRU87
Cholanthrene	(±)-3-MC-DE	C7-N2-dG	BBAI88

Table D.4.2-5: B[a]P-DNA Adducts Identified in Multiple Species

Metabolite	Adduct	Species	Strain	Reference
(+)-BPDE-II	C10-N2-dG	Mouse	SENCAR	JBAE90, JDIG85
			Swiss	JROJ86, JBAE81
			C57BL/6	JASH82, JKULK84
			A/HeJ	JSTO84, JADR83, JLUL84
		Rat	Wistar	JROJ86, JBAE81
		Hamster	Syrian	JMEL90
		Rabbit	NZ White	JSTO84
	C10-N6-dA	Mouse	SENCAR	JDIG85
		Rat	Wistar	JROJ86, JBAE81
(-)-BPDE-I	C10-N2-dG	Mouse	SENCAR	JDIG85
			Swiss	JROJ86, JBAE81
			A/HeJ	JSTO84, JADR83, JLUL84
			C57BL/6	JLUL84
		Rat	Wistar	JROJ86, JBAE81
(±)-BPDE-II	C10-N2-dG	Mouse	ICR	JRED90
			B6F3C1	JGIN90
(±)-BPDE-I	C10-N2-dG	Mouse	SENCAR	JBAE90, JDIG85
			Swiss	JROJ86
			A/HeJ	JSTO84, JADR83, JLUL84
			C57BL/6	JLUL84
		Rat	Wistar	JROJ86, JBAE81
		Rabbit	NZ White	JSTO84
B[a]P-9OH-4,5E	C4/C5-N2-dG	Mouse	SENCAR	JBAE90, JDIG85
			Swiss	JROJ86
		Rat	Wistar	JROJ86, JBAE81
Phenols	Unknown	Rat	S.D.	JBOR81
Quinones	Unknown	Rat	S.D.	JBOR81

As can be seen from the data in table D.4.2-5 many of the same B[a]P-DNA adducts are formed in a number of different species and strains within a species. While the data in table D.4.2-5 identifies the same B[a]P-DNA adducts in multiple species it does not consider the routes of administration. The following sections examine B[a]P-DNA adduct profiles in various tissues to determine if the route of administration has an effect on the adduct profile in a given tissue.

D.4.2.1.1 B[a]P-DNA Adduct Profiles

The following sections examine B[a]P-DNA adduct profiles in various tissues following topical, oral and inhalation administration. The object is to determine what effect, if any, the route of administration has on the types of adducts found in a given tissue. Where possible, comparisons will also be made between strains and species to identify any similarities of differences in adduct formation which may exist.

D.4.2.1.1.1 B[a]P-DNA Adduct Profiles: Topical Administration

Topical administration of B[a]P to various strains of mice results in the formation of the same adducts in the epidermis of each strain tested (see table D.4.2-6). The adduct profiles in all strains were very similar. The C10-N2-dG adduct of (+)-BPDE-II was the predominant adduct in all strains; accounting for between 70 and 90 percent of the total adducts in each case (see table D.4.2-6).

Two studies that have examined B[a]P adduct formation in Swiss mice report the major adduct peak as a mixture of adducts derived from both (+)-BPDE-II and (+)-BPDE-I in the female Swiss mouse and (+)-BPDE-II and (±)-BPDE-I in the male Swiss mouse (JROJ86,JBAE81). Studies with SENCAR mice have shown that (±)-BPDE-I-DNA adducts account for approximately 12 percent of total adduct formation (JDIG85). Based upon this observation, (±)-BPDE-I may be assumed to make approximately the same contribution to the total adduct levels in Swiss mice of both sexes. If one makes this assumption, the levels of (+)-BPDE-II-dG becomes 75 percent (78-12) in the female Swiss mouse and 73 percent (85-12) in the male Swiss mouse (see table D.4.2-6). The resultant (+)-BPDE-II-dG levels in the Swiss mice are comparable with those reported for SENCAR and C57BL/6 mice. Where reported, the levels of adducts derived from B[a]P-9OH-4,5E all fell within the same range (see table D.4.2-6). This data would suggest that strain differences in epidermal PAH-DNA adduct profiles are minimal. However, as was shown in Section D.4.1, total levels of adduct formation was greater in the SENCAR mouse than in the other strains.

The ratio of (+)-BPDE-II-dG adducts to the sum of (+) and (-)-BPDE-dA adducts is the same for the C57BL/6 (JASH82) and SENCAR (JDIG85) mouse strains. A similar comparison in male and female Swiss mice shows a two fold increase in the level of BPDE-II-dA adducts compared to the C57BL/6 or SENCAR steins (JROJ86,JBAE81). The level of dA adducts may be of interest as the suggestion of a correlation between the levels of dA adduct formation and the tumorigenic activity of a compound has been made in the literature (BBAI88,JBAE90,JCHA89,JJER91). Studies have shown that 1,2-diol-3,4-epoxide derivatives of DMBA and benzo[c]phenanthrene (B[c]Phen) exhibit a marked increase in dA binding compared to B[a]P (JCHA89,JJER91). At present however, it is difficult to fully assess the importance of this because the correlation between dA binding and tumorigenic activity of B[c]Phen is poor (JJER91). *In vivo*, the formation of the appropriate B[c]Phen-1,2-diol-3,4-epoxide is a very minor pathway in the metabolic processing of this compound (JJER91). Tumorigenicity studies using a substituted B[c]Phen that favours the formation of the 1,2-diol-3,4-epoxy derivative would be useful in further clarifying this issue.

The data in table D.4.2-6 is restricted to adducts found in epidermal tissue following topical application of B[a]P. Unfortunately, these studies have not examined the formation of DNA adducts in other tissue. Hughes and Phillips (JHUG90) have examined the formation of B[a]P-DNA adducts in the skin and lungs of male Parks mice following topical administration. Adduct levels in the skin reached a maximum 6 hours post treatment, while adduct levels in the lung peaked 48 hours after treatment. The maximum adduct levels in the skin were 6.5 fold higher than those reported in the lung (JHUG90).

Hughes and Phillips were able to identify five adducts in the skin following ^{32}P post labelling and thin layer chromatographic (TLC) analysis (JHUG90). Although individual adducts were not characterized, the relative contributions of each adduct peak to the total binding was estimated. Adducts 1,2 and 3 accounted for 97.5 percent of the total binding in mouse skin. In lung, the same three adducts accounted for 100 percent of the total binding (JHUG90) (see Figure D.4.2-1). Adduct 2, which accounts for approximately 75 percent of the total binding in both tissues is most likely (+)-BPDE-II-dG as this adduct consistently predominates in the other reported studies (see table D.4.2-6). Adduct 1 accounts for 20 and 23 percent of the total binding in skin and lung respectively (JHUG90). Tentative identification of this adduct is more difficult as other studies have not reported a specific study that consistently accounts for 20 percent of the total binding. Adducts derived from (±)-BPDE-I and B[a]P-9OH-4,5E have been reported as accounting for up to 13 percent of the identified adducts (see table D.4.2-6). Adduct 1 reported by Hughes and Phillips could be either one of these adducts, but without further information on which to base a comparison, a more definitive identification is not possible. There is insufficient information available to allow identification of the remaining adducts.

Table D.4.2-6: B[a]P-DNA Adduct Profiles in Epidermis Following topical Application to Various Strains of Mice

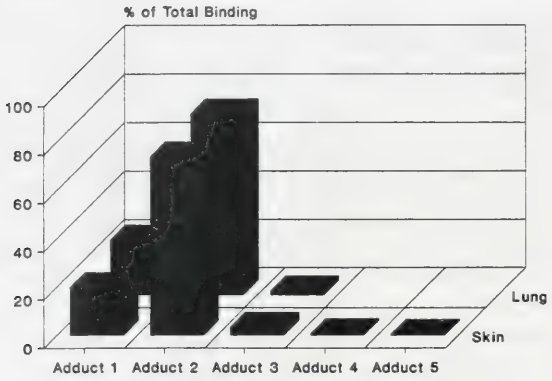
Strain (Sex)	Metabolite	Adduct	% Total[1]	Ref.
SENCAR (F)	(+)-BPDE-II	C10-N2-dG	73.1	JDIG85
		C10-N6-dA	1.7	JDIG85
	(-)-BPDE-II	C10-N2-dG	2.9	JDIG85
		C10-N6-dA	0.7	JDIG85
	(±)-BPDE-I	C10-N2-dG	12.6	JDIG85
		C10-N6-dA	0.7	JDIG85
	B[a]P-9OH-4,5E	C4/C5-N2-dG	5.9	JDIG85
	Unidentified	?	2.1	JDIG85
Swiss (F)	(+)BPDE-II (±)-BPDE-II	C10-N2-dG	87.1	JBAE81
	(+)-BPDE-II	C10-N6-dA	4.2	JBAE81
	(-)-BPDE-II	C10-N2-dG	5.8	JBAE81
	Unidentified	?	2.8	JBAE81
Swiss (M)	(+)-BPDE-II (+)-BPDE-I	C10-N2-dG	85.0	JROJ86
	(+)-BPDE-II	C10-N6-dA	4.4	JROJ86
	(-)-BPDE-II	C10-N2-dG	2.6	JROJ86
	B[a]P-9OH-4,5E	C4/C5-N2-dG	7.9	JROJ86
C57BL/6	(+)-BPDE-II	C10-N2-dG	72.6	JAHS82
	(±)-BPDE-II	C10-N6-dA	2.5	JAHS82
	B[a]P-9OH	?	10.8	JAHS82
	Unidentified	?	14.1	JAHS82

1 Percent of total identified adducts.

Topical administration of B[a]P to mice results in the formation of the same adducts in the epidermal tissue of all strains tested (see table D.4.2-6). The adduct profile in the lung, the only other tissue for which an adduct profile is available, differed from that in the skin only by the absence of some of the minor adducts (JHUG90). It is difficult to determine if the minor adducts were not present or just not detected. The total binding in the skin was 6.5 fold greater than that in the lung. Therefore, it is possible that minor adducts were present but not detected in the lung.

From the data presented, it would appear that topical administration of B[a]P results in the formation of the same adducts in multiple tissue. In addition, the adduct profiles would appear to be the same. However, the total levels of B[a]P-DNA adduct formation would appear to be lower in tissues removed from the site of application. It must be stressed that these conclusions are based upon a very small data set, with data in only two tissue (skin and lung). Therefore, the level of confidence in their validity must be low.

Figure D.4.2-1: **B[a]P-DNA Adducts in Skin and Lung Following Topical Application of B[a]P to Male Parkes Mice.** Values are given as percent total binding for each tissue.



D.4.2.1.1.2 B[a]P-DNA Adduct Profiles: Oral Administration

The formation of B[a]P-DNA adducts in multiple tissues following oral administration of B[a]P has been reported for four mouse strains; A/HeJ, ICR, BALB/c and C57BL/6 (JRED90, JSTO84, JADR83, JLUL84, JLU*90, JHUG90, JLU*86). Studies using A/HeJ mice have identified B[a]P-DNA adducts in lung, liver, forestomach, brain, colon and muscle (JSTO84,JADR83,JLUL84) (see table D.4.2-7). The adducts found in all tissue are similar to those found in the skin following topical application. In addition, the adduct profiles are similar (see table D.4.2-6 and 4.2-7 and figure D.4.2-2). In all tissues examined, (+)-BPDE-II-dG was the predominant adduct found, representing between 67 and 95 percent of the adducts identified (JSTO84,JADR83,JLUL84). It should be noted that the "%Total" values presented in table D.4.2-7 are percent totals for the individual tissues examined and not a percent of the total administered dose. Adducts derived from (±)-BPDE-I were the second most abundant adducts found, accounting for between 10 and 16 percent of the total adducts in all tissues but the colon. In the colon, adducts derived from (-)-BPDE-II were three to four fold more abundant than those derived from (±)-BPDE-I (JSTO84). The reason for this is not clear. Adducts derived from (-)-BPDE-II were found in all tissues examined while unidentified adducts were detected only in the lung, liver and forestomach (JSTO84).

The dose dependency of B[a]P-DNA adduct tissue distribution was examined by Stowers *et al.* (JSTO84). Figures D.4.2-3 and D.4.2-4 outline the adduct profiles in the various tissue tissues examined following oral administration of B[a]P at low (11.9 µmoles/Kg) and high (1190 µmoles/kg) doses. The data are presented as pmoles adduct bound per mg DNA (JSTO84). At both doses the (+)-BPDE-II-dG adduct predominates in all tissues, accounting for 80 and 75 percent of the total adduct detected respectively (JSTO84).

At the low dose (+)-BPDE-II and (±)-BPDE-I adducts were reported in all tissues examined with the highest levels found in the lung, liver and forestomach. Adducts derived form (-)-BPDE-II were identified in all tissues except the liver (JSTO84) (see figure D.4.2-3). The reason for this is unclear, but may be due, in part, to increase Phase II conjugation reactions in the liver compared to

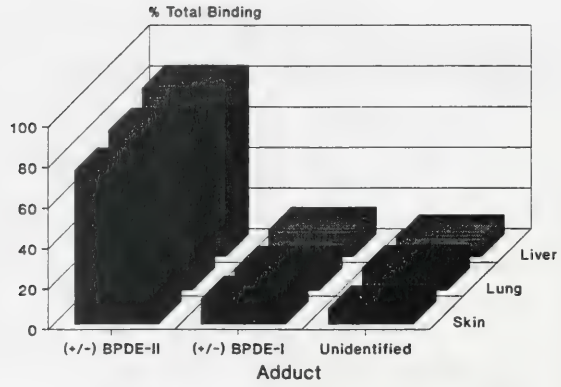
other tissues (see Section D.3). The unidentified adduct peak was found only in the lung (JSTO84). Again, the reason is unclear. The adducts in the *"Unidentified"* peak, although not fully characterized, are thought to be derived from either B[a]P-9OH-4,5E or (+)-BPDE-II-dC (JSTO84).

Table D.4.2-7: B[a]P-DNA Adduct Profiles in A/HeJ mice Following Oral Administration of B[a]P

Tissue	Adduct	% Total[1]	Ref.
Lung	(+)-BPDE-II-dG	71.8±2.1	JSTO84, JADR83, JLUL84
	(-)-BPDE-II-dG	7.1±1.6	JSTO84, JADR83, JLUL84
	(±)-BPDE-I-dG	10.9±2.2	JSTO84, JADR83, JLUL84
	Unidentified	11.3±1.7	JSTO84, JADR83, JLUL84
Liver	(+)-BPDE-II-dG	75.3±8.7	JSTO84, JADR83, JLUL84
	(-)-BPDE-II-dG	7.8	JADR83, JLUL84
	(±)-BPDE-I-dG	13.3±2.1	JSTO84, JADR83, JLUL84
	Unidentified	9.5	JADR83, JLUL84
Kidney	(+)-BPDE-II-dG	83.8	JSTO84
	(-)-BPDE-II-dG	9.0	JSTO84
	(±)-BPDE-I-dG	7.2	JSTO84
Forestomach	(+)-BPDE-II-dG	89.3	JSTO84, JADR83
	(-)-BPDE-II-dG	6.2	JSTO84
		Trace	JADR83
	(±)-BPDE-I-dG	7.6	JSTO84
	Unidentified	trace	JADR83
Brain	(+)-BPDE-II-dG	76.4	JSTO84
	(-)-BPDE-II-dG	7.0	JSTO84
	(±)-BPDE-I-dG	16.6	JSTO84
Colon	(+)-BPDE-II-dG	85.0	JSTO84
	(-)-BPDE-II-dG	11.1	JSTO84
	(±)-BPDE-I-dG	3.9	JSTO84
Muscle	(+)-BPDE-II-dG	82.8	JSTO84
	(-)-BPDE-II-dG	7.7	JSTO84
	(±)-BPDE-I-dG	9.5	JSTO84

1 Values are % total binding in each tissue not % total dose

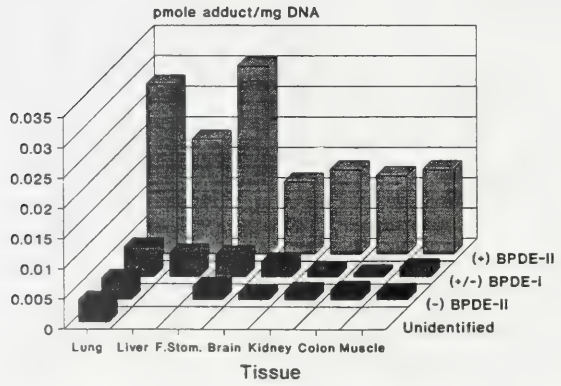
Figure D.4.2-2: B[a]P-DNA Adduct Profiles in Skin[1], Liver[2] and Lung[2]



1 Adduct profiles in skin following topical application

2 Adduct profiles in liver and lung following oral administration.

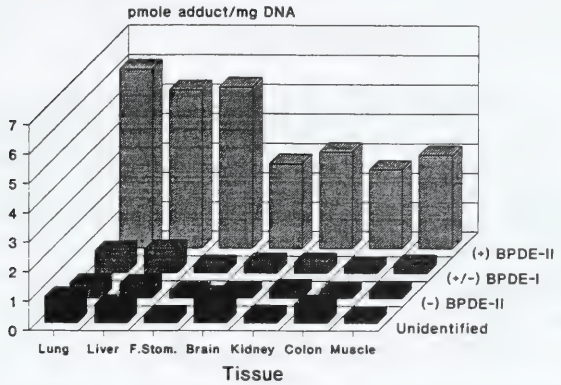
Figure D.4.2-3: B[a]P-DNA Adduct Tissue Profiles in A/HeJ mice Following Oral Administration (Low Dose = 11.9 μmoles/Kg)



At the high dose, all four adduct peaks were identified in all tissue (see figure D.4.2-4). Again, adducts derived from (+)-BPDE-II were the major adducts detected (JSTO84). All three adducts derived from BPDE showed essentially the same tissue profiles.

The data reported by Stowers *et al.*indicate that the formation of B[a]P-DNA adducts is dose-dependent and that increasing dose results in increases in B[a]P-DNA adduction. It is difficult to determine the nature of this dependence based upon the information provided by Stowers *et al.* (JSTO84). A one hundred fold increase in dose led to an average 200 fold increase in BPDE adduct formation.

Figure D.4.2-4: **B[a]P-DNA Adduct Tissue Profiles in A/HeJ mice Following Oral Administration (High Dose = 1190 μmoles/Kg)**



Oral administration of B[a]P results in the formation of B[a]P-DNA adducts in numerous tissues (see figures D.4.2-3 and D.4.2-4). The adducts identified are the same as those found in the lung and skin following topical application. In all tissues examined, (+)-BPDE-II-dG was the predominant adduct formed. In addition, adduct profiles are approximately the same in all tissues for which data is available. Total adduct levels were highest in the tissues first exposed to B[a]P eg; forestomach, liver and lung. Contrary to expectation, adduct levels in the lung are higher than those in the liver following oral administration despite liver being exposed to B[a]P prior to the lung. The increased adduct levels may be due to the transport of reactive metabolites from the liver to the lung. It may also be due to an increased level of Phase II enzyme activities in the liver compared to the lung (see Section D.3.3).

The data presented would suggest that adduct profiles following oral administration are similar in all tissues for which data are available. Total adduct levels tend to be lower in tissue removed from the site of administration. It should be stressed that limited data is available. Therefore, the confidence in the validity in the observation should be low.

D.4.2.1.1.3 B[a]P-DNA Adduct Profiles: Inhalation Administration

Studies which report tissue B[a]P-DNA adduct profiles could not be located int the literature.

D.4.2.1.2 Formation of Specific B[a]P-DNA Adducts: Summary of Conclusions

D.4.2.1.2.1 Topical Administration: Summary

Topical administration of B[a]P results in the formation of similar B[a]P-DNA adducts in epidermal tissue in multiple strains of mice. Comparisons are hampered however by incomplete identification of all adducts. In order to make adequate comparisons, there is a need for studies which provide a more detailed identification of all B[a]P-DNA adducts in more than one strain of mouse. In addition, studies which examine adduct profiles in more than one tissue would aid in increasing the confidence in the comparisons of routes of administration.

D.4.2.1.2.2 Oral Administration: Summary

There is limited data which reports adduct levels in a wide range of tissues in a number of strains (see Section D.4.2.1.1.2). The data indicate that the strains studied all show similar tissue adduct profiles. While there is sufficient data to do this type of comparison, there are limitations to this approach. It is not possible, in many cases, to determine what the absolute levels of binding are. It would be extremely useful to have information on total adduct levels for all of the strains and species studied. This would facilitate the correlation between adduct profiles and tumorigenic response. At present, all that can be stated is; that for the strains investigated, the pattern of adduct distribution throughout the body are similar.

D.5 Summary of Conclusions

D.5.1 Grouping PAH

D.5.1.1 Are All PAH Metabolized By The Same Enzymes?

The binding of reactive PAH metabolites to cellular macromolecules, in particular DNA, has the potential to lead to a tumorigenic response (8). There is a wide range of tumorigenic potencies noted among PAH (see Main Document). If the PAH which show high tumorigenic potency are metabolized by a different group of enzymes than those which produce little or no tumorigenic response, it may be possible to predict the metabolic/tumorigenic behaviour of PAH based on the enzymes responsible for their metabolism. The following section examines the enzymes responsible for PAH metabolism.

D.5.1.1.1 Available Data

Unlike other sections where data are limited, a large amount of information exists on the metabolism of PAH and the enzymes involved therein (see Section D.1). The following is a very brief summary of the information contained in Section D.1 and Section D.2.

D.5.1.1.2 Discussion and Conclusions

The wealth of available literature indicates that all PAH are indeed metabolized by the same group of enzymes (see Section D.1). While the metabolic profile of each PAH is dependent on the primary metabolites formed by cytochrome P-450 there are a few generalizations which can be made.

i) Most PAH require cytochrome P-450 oxidation as an initial metabolic step before they are sufficiently reactive to be processed by either E.H. or GST. Cytochrome P-450 introduces an atom of oxygen across an aromatic C=C double bond on the periphery of the PAH molecule. The resultant primary epoxide is the open to a number of metabolic processes.

ii) Once a primary epoxide has been formed, it is open to a number of metabolic reactions, each of which leads to a different product (see Section D.1). Epoxide hydrolase further oxidizes the PAH by converting the primary epoxide to a dihydrodiol. The dihydrodiol is then subject to either further oxidation by cytochrome P-450 to produce diol-epoxides and tetrols, or to conjugation by either UDP-GT or ST for subsequent removal (see Section D.1 and Section D.2). The primary epoxide is also open to conjugation by GST to produce glutathione derivatives which are excreted. Non-enzymatic rearrangement of the epoxide to produce phenolic derivatives are also possible. The location of the primary epoxide in the parent ring governs what products are subsequently produced (see Section D.1).

iii) Some substituted PAH are sufficiently reactive to undergo conjugation without prior cytochrome P-450 activation. Some amino and nitro substituted PAH are able to undergo conjugation with UDP-GT to form glucuronide amino and nitro derivatives. These products are formed without modification of the parent ring structure. While these compounds are able to sidestep Phase I metabolism, many still pass through Phase I reactions prior to conjugation (see Section D.1).

From the data presented here and in Sections D.1 and D.2 it would seem that grouping PAH based on the enzymes responsible for their metabolism will not be possible.

D.5.1.2 Are Similar Metabolites Formed For All PAH?

The tumorigenic action of PAH is though to be related to their ability to bind to cellular macromolecules including DNA (see Section D.1 and Section D.4). The number of reactive metabolites formed from a given PAH may play a part in explaining the observed differences in tumorigenic potencies of PAH (see Main Document). This section examines the metabolic profiles of a number of PAH to determine what types of metabolites are formed for each, and to determine if the number of reactive metabolites formed can be correlated with the tumorogenic potencies of various PAH.

D.5.1.2.1 Available Data

There is a large amount of data available on the metabolism of PAH. The majority of the work comes from either cell culture or microsomal preparations. The main focus has been the identification of Phase I metabolic products, as it is these which generally produce reactive metabolites (see Section D.1). The conjugation products of Phase II metabolism have received less attention as these compounds are usually excreted and are seldom subject to further metabolic activation. Therefore, this section will focus on the products of Phase I metabolism.

D.5.1.2.2 Results

When comparing the Phase I metabolic profiles of numerous PAH, it is essential to choose a metabolic system which is common to the largest number of compounds. The most commonly cited system is the Rat Liver Microsomal (RLM) system. Therefore in selecting data for this section, preference was given to data derived from RLM studies. In addition, preference was given to studies which used "control" of non-induced RLM systems. While it is possible that the metabolite profile in control and induced systems may differ marginally, a cytochrome P-450 inducer common to all PAH studied could not be found. Therefore it was felt that the control RLM system offered the largest data set on which comparisons could be made. The data on Phase I PAH metabolites is summarized in table D.5.1-1.

Table D.5.1-1: Metabolite Profiles of Various PAH in the Control RLM System

Parent	Metabolite	Region	% Total	Ref
Dibenz[a,j]acridine	Tetrols			JROB90
	5,6-diol	K-region		JROB90
	5,6-epoxide	K-region		JROB90
	3,4-diol	DEP1		JROB90
	3-hydroxy			JROB90
	4-hydroxy			JROB90
Dibenzo[a,e]pyrene	11,12-diol	Bay/DEP1		JDEV90
	11-hydroxy			JDEV90
	1-hydroxy			JDEV90
	3-hydroxy			JDEV90
Dibenzo[a,l]pyrene	8,9-diol	K-region		JDEV90
	11,12-diol	DEP1		JDEV90
	7-hydroxy			JDEV90
	Diones			JDEV90
Phenanthrene	1,2-diol	DEP1	14%	JLEV82 BSIM81
	3,4-diol	Bay	2%	JLEV82 BSIM81
	9,10-diol	K-region	77%	JLEV82 BSIM81
	Phenols		8%	JLEV82 BSIM81
3-Methylcholanthrene	1-hydroxy			BSIM81
	3-hydroxy			BSIM81
	3-hydroxymethyl			BSIM81
	11,12-diol	K-region		BSIM81
Dibenz[a,h]anthracene	1,2-diol	Bay	8%	JLEV82 BSIM81
	3,4-diol	DEP1	26%	JLEV82 BSIM81
	5,6-diol	K-region	2%	JLEV82 BSIM81
	Phenols		47%	JLEV82
	Unidentified		17%	JLEV82

Table D.5.1-1 continued on next page

Parent	Metabolite	Region	% Total	Ref
Dibenz[a,c]anthracene	10,11-diol	Bay	Major	BSIM81
	1,2-diol	Bay		BSIM81
	3,4-diol	Bay		BSIM81
Benzo[c]phenanthrene	1,2-diol	Bay	<1%	JLEV82
	3,4-diol	DEP1	15%	JLEV82
	5,6-diol	K-region	75%	JLEV82
	Phenols		10%	JLEV82
Chrysene	1,2-diol	DEP1	34%	JLEV82 BSIM81
	3,4-diol	Bay	35%	JLEV82 BSIM81
	5,6-diol	K-region	2%	JLEV82 BSIM81
	Phenols		17%	JLEV82
	Unidentified		13%	JLEV82
5-Methylchrysene	1,2-diol	DEP1	Major	BSIM81
	7,8-diol	DEP1	Major	BSIM81
	5-hydroxymethyl			BSIM81
	Phenols			BSIM81
7-Methylbenz[a]anthracene	1,2-diol	Bay		BSIM81
	3,4-diol	DEP1		BSIM81
	5,6-diol	K-region		BSIM81
	8,9-diol	DEP2		BSIM81
	10,11-diol	Bay		BSIM81
	7-hydroxymethyl			BSIM81
7,12-Dimethylbenz[a]anthracene	7-(CH_2OH)-12-methyl			BSIM81 JMEY89
	12-(CH_2OH)-7-methyl			BSIM81 JMEY89
	7,12-di(CH_2OH)			BSIM81
	5,6-diol-12-(CH_2OH)	K-region		BSIM81
	8,9-diol-7,12-di(CH_2OH)			BSIM81

Table D.5.1-1 continued on next page

Parent	Metabolite	Region	% Total	Ref
Benz[a]anthracene	5,6-diol	K-region	44%	JLEV82 BSIM81 BJAC82 JMUS86
	8,9-diol	DEP2	42%	JLEV82 BSIM81 BJAC82 JMUS86
	3,4-diol	DEP1	4%	JLEV82 BSIM81
	10,11-diol	DEP2	4%	JLEV82 BSIM81 BJAC82 JMUS86
	1,2-diol	Bay	<1%	BSIM81 JLEV82
	1-hydroxy			BJAC82 JMUS86
	2-hydroxy			JMUS86
	4-hydroxy			BJAC82 JMUS86
	5-hydroxy			BJAC82
	8-hydroxy			BJAC82 JMUS86
	9-hydroxy			JMUS86
	10-hydroxy			JMUS86
	11-hydroxy			BJAC82 JMUS86
	Phenols		6%	JLEV82
Pyrene	1-hydroxy		Major	BJAC82
	4,5-diol	K-region		BJAC82
	Diphenols			BJAC82
	non-K-region triols			BJAC82
Fluoranthene	2,3-diol	non-K	37%	JBAB86
	2,3-quinone		13%	JBAB86
	1-hydroxy		10%	JBAB86
	3-hydroxy		8%	JBAB86
	8-hydroxy		9%	JBAB86

Table D.5.1-1 continued on next page

Parent	Metabolite	Region	% Total	Ref
Benzo[e]pyrene	4,5-diol	K-region		BSIM81 BJAC84
	9,10-diol	Bay		BSIM81 BJAC84
	1-hydroxy			BJAC84
	3-hydroxy			BJAC84
Benzo[a]pyrene	4,5-diol	K-region	9%	JLEV82
	7,8-diol	DEP1	10%	JLEV82
	9,10-diol	Bay	14%	JLEV82
	11,12-diol	Bay	0	JLEV82
	Quinones		35%	JLEV82
	Phenols		33%	JLEV82

In most cases, the ultimate active metabolite is a diol-epoxide which is capable of reacting with biological macromolecules (see Section D.1 and Section D.4). Interest in PAH metabolites must therefore attempt to focus on the formation of these compounds. However, these compounds are relatively unstable and are difficult to isolate and identify in biological system. More commonly either the precursors or products of these structures are identified. Therefore in table D.5.1-1 metabolites which can serve as diol-epoxide precursor have been identified. The dihydrodiols which serve as diol-epoxide precursors have been identified as being formed in the "Diol-Epoxide Precursor" Region (DEP) and are indicated in the table as such.

Two types of diol-epoxide precursors have been identified in table D.5.1-1. There are;

DEP1: These are diol-epoxide precursors on benzene ring immediately adjacent to the bay region. Eg. B[a]P-7,8-diol.

DEP2: These are diol-epoxide precursors which are removed from the bay region y at least one fused ring. Eg. Benz[a]anthracene-8,9-diol.

Other abbreviations used in table D.5.1-1 include;

K-region: These are epoxides and dihydrodiols located in the K-region.

Bay: These are metabolites formed immediately in the Bay-region.

Diol: Compounds identified in this manner are dihydrodiols.

(CH_2OH): Compounds identified in this manner are hydroxymethyl compounds.

D.5.1.2.3 Discussion and Conclusions

From the data presented in table D.5.1-1 it can be clearly seen that the same types of Phase I metabolites are formed for all PAH for which data are reported. What differs is the location of the oxidation on the ring structure. It should be noted that while diol-epoxide precursors are formed in a number of PAH not all of these are subsequently converted to the corresponding diol-epoxide. In addition diols which in table D.5.1-1 are listed as being in the Bay-region generally do not go on to form diol-epoxides. The biologically reactive diol-epoxides have the epoxide located in the bay-region (see Section D.1 and Section D.4). It is true that compounds which have a diol in the bay-region can form diol-epoxides, but these compounds are very rare.

From the data presented in table D.5.1-1 it can be seen that a number of PAH are metabolized to DEP1 compounds. For a number of these, the metabolite profiles have been reported. This data is summarized in table D.5.1-2. The data in table D.1.3-1 shows that DEP1 compounds range from a low of 4 percent of the total metabolites formed for benz[a]anthracene to a high of 34 percent for chrysene. Initial thought would suggest that compounds which produce a greater percentage of DEP1 compounds would be more tumorigenic than those which produce moderate or low levels of DEP1 metabolites. This however is not the case. Chrysene for which the 1,2-diol accounts for 34 percent of the total metabolites formed is only weakly tumorigenic, while B[a]P which produces only 10 percent is a potent PAH tumorigen (see Main Document). Clearly factors other than the total level of DEP1 formed are important in the generation of the ultimately reactive metabolite.

Table D.5.1-2: Percent of Diol-Epoxide Precursor 1 Formed for Various PAH

Compound	% of Total
Phenanthrene, 1,2-dihydroxy-1,2-dihydro-, (±)	14
Benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-, (±)	4
Dibenz[a,h]anthracene, 3,4-dihydroxy-3,4-dihydro-, (±)	26
Benzo[c]phenanthrene, 3,4-dihydroxy-3,4-dihydro-, (±)	15
Chrysene, 1,2-dihydroxy-1,2-dihydro-, (±)	34
5-Methylchrysene, 1,2-dihydroxy-1,2-dihydro-, (±)	Major
Benzo[a]pyrene, 7,8-dihydroxy-7,8-dihydro-, (±)	10

The rate a which the DEP1 is converted to a diol-epoxide will be one of the most important factors in correlating tumor potency with metabolite production. While the data presented in tables D.5.1-1 and 5.1-2 indicates the amount of DEP1 formed for a number of PAH, what it does not provide is the stereochemical breakdown of these compounds. There are four B[a]P-7,8-diol-9,10-epoxides compounds possible each of which has specific stereochemistry. The stereochemical arrangement of the epoxide is critical in determining a compounds ability to bind to DNA (see Section D.4). Therefore in order to properly address the issue of differing potencies based on metabolite profiles a good base of metabolite profile at the stereochemical level is needed for a number of PAH with varying degrees of tumor potency.

D.5.1.3 What Structural Features Govern The Metabolic Processing of PAH?

The main reason for asking this question is to determine if it is possible to predict the metabolic and tumorigenic activity of a PAH based upon its chemical structure. Of primary concern is the formation of PAH-DNA adducts which is thought to be an essential step in the tumorigenic process (see Section D.4). Therefore it would be advantageous to be able to predict if a given PAH has the potential to be converted to metabolites capable of binding to DNA.

The types of PAH-DNA adducts which have been identified have been reviewed in Section D.4.2. For the majority of PAH for which PAH-DNA adducts have been reported, the predominant adducts are derived from bay-region diol-epoxides (see table D.5.1-3). The "Bay-Region Theory" has been used to predict the carcinogenicity of PAH (JLEV82). This theory postulates that epoxides on saturated angular benzo-rings, when present in the bay-region of a PAH, should possess uniquely high chemical reactivity (JLEV82). Based on the assumption that high reactivity correlates with high biological activity, the bay-region theory suggests that bay-region diol-epoxides are the ultimate carcinogenic metabolites of PAH (JLEV82). As can be seen from the list of adducts in table D.5.1-3, bay-region diol-epoxides are not solely responsible for the formation of PAH-DNA adducts. However, it can also be seen that bay-region diol-epoxides are the predominant form of PAH-DNA adducts formed.

Table D.5.1-3: PAH Metabolites Identified as PAH-DNA Adducts *In Vivo*

Metabolite	Reference
Benz[a]anthracene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	BBAI88
Benz[a]anthracene, 8ß,9α-dihydroxy-10α,11α-epoxy-8,9,10,11-tetrahydro-, (±)	BBAI88
Benz[a]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)	BBAI88, JDIP90
Benz[a]anthracene, 3ß,4α-dihydroxy-1α,2α-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)	BBAI88, JDIP90
Benz[a]anthracene, 5,6-dihydro-5,6-epoxy-, (±)	JCHA89
Benzo[a]pyrene, 7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+)	JDIG85, JROJ86
Benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (-)	JDIG85, JROJ86
Benzo[a]pyrene, 7ß,8α-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahdryo-, (±)	JDIG85, JROJ86
Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-9-hydroxy-, (±)	JDIG85
Benzo[a]pyrene, 3-hydroxy-	JBOR81
Benzo[c]phenanthrene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	JCHA89, JPRU87
Benzo[c]phenanthrene, 3α,4ß-dihydroxy-1α,2 -epoxy-1,2,3,4-tetrahydro-, ( )	JCHA89, JPRU87
Chrysene, 1ß,2 -dihydroxy-3 ,4 -epoxy-1,2,3,4-tetrahydro-, ( )	BBAI88
Chrysene, 1ß,2 ,9-trihydroxy-3 ,4 -epoxy-1,2,3,4-tetrahydro-, ( )	BBAI88
Cholanthrene, 9ß,10 -dihydroxy-7 ,8 -epoxy-3-methyl-7,8,9,10-tetrahydro-, ( )	BBAI88

The bay-region theory does not attempt to include stereochemical or metabolic factors when predicting carcinogenicity. The importance of these factors can be seen if one examines the carcinogenic potencies of a number of PAH (see table D.5.1-4).The data in table D.5.1-4 show a large variation in potencies between compounds which can form diol-epoxides. For example; benzo[a]pyrene (Relative Potency (RP) = 1) which has a single bay-region is approximately 10 fold more potent than chrysene (RP = 0.12) which has two bay-regions. Clearly the presence of a bay-region is not sufficient to impart tumorigenic behaviour to a compound. The way in which a compound is metabolized and the products formed are critical factors in conferring tumorigenic potential on a PAH. The formation of diol-epoxides from dihydrodiols is highly stereospecific (JLEV82). Therefore, the formation of diol-epoxides capable of binding to DNA is dependent upon the stereochemical configuration of the starting dihydrodiol. Any scheme which attempts to group PAH must account for the metabolic behaviour of a PAH and predict the stereochemical breakdown of the products formed.

At present it is difficult to predict the metabolic behaviour of PAH. Studies which have attempted to predict metabolic behaviour by using molecular orbital calculations, have met with only modest success (JLEV82). It is perhaps possible to suggest that compounds which possess a bay-region are more likely to show tumorigenic activity than compounds which do not have bay-regions. Prediction beyond this is not possible with our present understanding of PAH metabolism.

D.5.2 Species Differences

Differences in species sensitivity to the tumorigenic effects of PAH may be due, in part, to differences in the levels of reactive PAH metabolites formed in each species. A very simplistic model would suggest that a species in which the formation of reactive metabolites is greatest should demonstrate the greatest sensitivity to PAH tumorigenicity.

D.5.2.1 Are Enzyme Activities The Same In Different Species?

This section examines enzyme tissue profiles data in the rat and the mouse to determine if differences in enzyme activities between the species can be correlated with differences in sensitivity to PAH tumorigenicity.

Table D.5.1-4: Relative Potencies of Selected PAH in CD-1 Mice (Main Document)

Compound	Relative Potency
Benzo[a]pyrene	1.00
Cholanthrene, 3-methyl-	4.67
Benz[a]anthracene, 7,12-dimethyl-	2.0
Dibenz[a,h]anthracene	1.63
Chrysene	0.12
Benzo[c]phenanthrene	0.08
Benz[a]anthracene	0.06
Pyrene	0.009
Benzo[e]pyrene	0.003

D.5.2.1.1 Available Data

There is a limited amount of enzyme tissue profile data available in the literature (see Section D.3). The majority of the data examines enzyme tissue profiles in the rat. There are minimal amounts of enzyme tissue profile data available in the mouse, and none for other species. As a result of this shortage of data, the level of confidence in the conclusions drawn herein must be low.

D.5.2.1.2 Results

Enzyme levels in both rat and mouse are shown in tables D.5.2-1 and D.5.2-2. The rat enzyme tissue profile data was taken from Section D.5.3.2 and the derivation of the levels reported in table D.5.3-3 can be found therein. The enzyme tissue profile data for the mouse was derived principally from two reports (JAKI76,JOES77). A detailed discussion of the mouse enzyme tissue profile data can be found in Section D.3.2.2 and Section D.3.3.3.

Akin *et al.* (JAKI76) reported AHH activity in liver, lung and epidermis in ICR and C57BL/6 mice. The differences in AHH activity were sufficiently small that it was feasible to average the strains (see Section D.3.2.2 and table D.3.2-14). The resulting AHH activities are shown in table D.5.2-2. Pohl *et al.* reported hepatic AHH activity of 1.310 nmole/min/mg microsomal protein in CD-1 mice. There is insufficient data to determine if the large difference in AHH activity between the CD-1 and ICR and C57BL mice is due to strain differences or due to other factors. Consequently the data for CD-1 mice has been excluded from the present consideration. In mouse, EH activity is reported in a number of strains. However, only one study (JOES77) used the same substrate as that used to measure EH activity in the rat (B[a]P-4,5-epoxide). Therefore this study was chosen for comparisons with rat EH activity.

Table D.5.2-1: Tissue Enzyme Profiles in the Rat

	Tissue (Activity = nmoles product formed/min/mg protein)							
Enzyme	**Liver**	**Lung**	**Kidney**	**Whole Skin**	**Testis**	**Stomach**	**Intestine**	**Ref.**
AHH	0.295±0.07	0.01±0.007	0.005±0.002	0.004	0.002±0.001	0.001	0.001	*Section D.5.3.2*
EH	6.66±0.3	0.35±0.02	0.73±0.02	0.12	1.47	0.14	0.13	*Section D.5.3.2*
GST	763±204	59	193±7					*Section D.5.3.2*

Table D.5.2-2: Tissue Enzyme Profiles in the Mouse

	Tissue (Activity = nmoles product formed/min/mg protein)							
Enzyme	**Liver**	**Lung**	**Kidney**	**Whole Skin**	**Testis**	**Epidermis**	**Intestine**	**Ref.**
AHH	0.75	0.02				0.009		JAKI76
EH	0.90	0.43	0.07	0.17	2.19		0.03	JOES77
GST	4244±768							JBOR91

D.5.2.1.3 Discussion and Conclusions

The data presented in table D.5.2-1 and D.5.2-2 suggest that AHH activity in the liver and lung of the mouse are approximately 2 fold greater than those in the rat. Epoxide hydrolase levels in mouse hepatic tissue is significantly lower than that found in rat hepatic tissue. In all other tissues except testis, E.H. levels in the rat and mouse are comparable. Epoxide hydrolase levels in mouse testis are higher than those in mouse liver, a pattern not seen in the rat. A more detailed discussion of E.H. tissue levels can be found in Section D.3.3.3. Glutathione-S-transferase levels in mouse liver are approximately 5.6 fold greater than those reported in rat hepatic tissue. There is insufficient data to draw any concrete conclusions from the tissue levels data presented above.

In the rat, hepatic E.H./AHH and GST/AHH ratios are quite large; approximately 20 fold and 2300 fold respectively. In the mouse however, the hepatic E.H./AHH ratio is much smaller; 1.2 fold, and the GST/AHH ratio is much larger; 12600 fold, than those seen in the rat. In order to understand how these differences can be related to differences in sensitivity to PAH, it is necessary to know something about the reactions carried out by these enzymes. Section D.2 provides a review of the specificity and mechanism of action for all the major enzymes involved in PAH metabolism.

Cytochrome P-450c (AHH) is responsible for the production of primary epoxides and diol-epoxides. Epoxide hydrolase converts epoxides to dihydrodiols. Primary epoxides serve as very good substrates for E.H.. However, diol-epoxides are poor substrates (JOES83),(see Section D.2.1.2.3). Glutathione-S-transferase is also able to remove primary epoxides by direct conjugation of the epoxide (JGRO86, JNEM81) (see Section D.2.2.1.3). Epoxides formed in the K-region serve as good substrate for both E.H. and GST. Non-K-region epoxides and diol-epoxides are poorer substrates (see Section D.2.2.1.3). Using this information and the data presented above, it may be possible to provide a partial explanation of the observed species differences in sensitivity to the tumorigenic effects of PAH (see Main Document).

In the rat, AHH levels are significantly lower than those of either E.H. or GST. therefore, it is possible that any epoxides or diol-epoxides produced by AHH are easily removed by either E.H. or GST. As noted above, diol-epoxides are poor substrates for both E.H. and GST. However, the high levels of both enzymes should insure that most diol-epoxides are removed before they are able to interact with and bind to DNA. In the mouse, AHH levels are 2.2 fold greater than those in the rat. Therefore it is to be expected that the production of primary epoxides and diol-epoxides would also be greater than that seen in the rat. At the same time, E.H. levels are only 13.5 percent of those seen in the rat. In addition, GST levels in the mouse are only 5.6 fold greater than those in the rat. The reduction in the E.H. ratio in the mouse compared to that in the rat may be, in part, responsible for the increase sensitivity seen in the mouse. The low E.H./AHH ratio may mean that the diol-epoxides formed by AHH activity are removed more slowly than those in the rat. The result could be an increased potential for diol-epoxide interaction with DNA. The high levels of GST in mouse hepatic tissue could be expected to remove the majority of the diol-epoxides formed by AHH. However, this would depend on the affinity of the GST for the diol-epoxide. These differences in enzyme levels between the two species may, in part explain the observed differences in sensitivity to PAH tumorigenicity. Differences in the affinity of each enzyme for the substrate between the species may also play an important role.

From the data presented, it would appear that enzyme activities differ between the mouse and the rat. In addition, these differences may play a role in the differing sensitivities to PAH observed between the species. It must be stressed that these conclusions are based on very small data sets. Therefore the confidence placed in the conclusions must be low. Further data on enzyme levels in multiple tissues in more than one species, and examinations of differences in affinities between species is needed before firmer conclusions can be drawn.

D.5.2.2 Are PAH Metabolite Profiles The Same In Different Species?

This section examines metabolite profiles in various species to determine if differences in the types and levels of metabolites formed can be correlated with differences in sensitivity to PAH tumorigenicity.

D.5.2.2.1 Available Data

There exists a reasonable amount of data for the metabolism of various PAH using the rata liver microsomal system (RLM). However, metabolite profile data in other species appear to be limited. In addition, true *in vivo* data is extremely limited. All data examines PAH metabolism in liver microsomal systems. Two studies were judged to contain data adequate for addressing the question of PAH metabolite profiles in multiple species. The data from these studies is discussed below.

D.5.2.2.2 Results

Selkirk *et al.* (JSEL77) reported metabolite profiles in rat, mouse and hamster liver microsomes. The data showed that many of the same metabolites were found in all three systems (see table S.5.2-3). However, the levels of each metabolite varied from species to species. The formation of metabolites in each species was monitored with a starting with 100 nmoles B[a]P; therefore direct comparison between the three species is possible. In each species, the majority of B[a]P remained as unmetabolized B[a]P at the end of the 30 minute incubation periods.

Table D.5.2-3: Metabolite Profiles for B[a]P in Rat, Mouse and Hamster Liver Microsomes (JSEL77).

	Rat	Mouse	Hamster
Metabolite	**pmoles**	**pmoles**	**pmoles**
B[a]P-4,5-dihydroxy-4,5-dihydro- (±)	90	58	536
B[a]P-7,8-dihydroxy-7,8-dihydro- (±)	136	250	91
B[a]P-9,10-dihydroxy-9,10-dihydro- (±)	275	154	100
B[a]P-3-hydroxy-	600	1394	800
B[a]P-9-hydroxy-	112	442	36
B[a]P-diones	161	192	

Jacob *et al.* reported benzo[e]pyrene metabolite profiles in rat, mouse and human liver microsomes (BJAC84). As Selkirk found for B[a]P (JSEL77), Jacob *et al.* showed that the same metabolites were formed in all species (see Table D.5.2-4). The levels of the individual metabolites formed in each species differed (see Figure D.5.2-1).

Table D.5.2-4: Metabolite Profiles for B[e]P in Rat, Mouse and Human Liver Microsomes (BJAC84)

	Rat		Mouse		Human	
Metabolites	**nmoles**	**R.L.[1]**	**nmoles**	**R.L.**	**nmoles**	**R.L.**
B[e]P-1-hydroxy-	0.6	50	2	53	1.4	22
B[e]P-3-hydroxy-	0.4	33				
B[e]P-4,5-diol[2]	1.2	100	3.8	100	6.4	100
B[e]P-9,10-diol[2]					0.8	13

1) Relative product levels. Levels were set relative to the metabolite produced in greatest quantity (see text for discussion).

2) Benzo[e]pyrene, 4,5-dihydroxy-4,5-dihydro (±) and Benzo[e]pyrene 9,10-dihydro-9,10-dihydro (±).

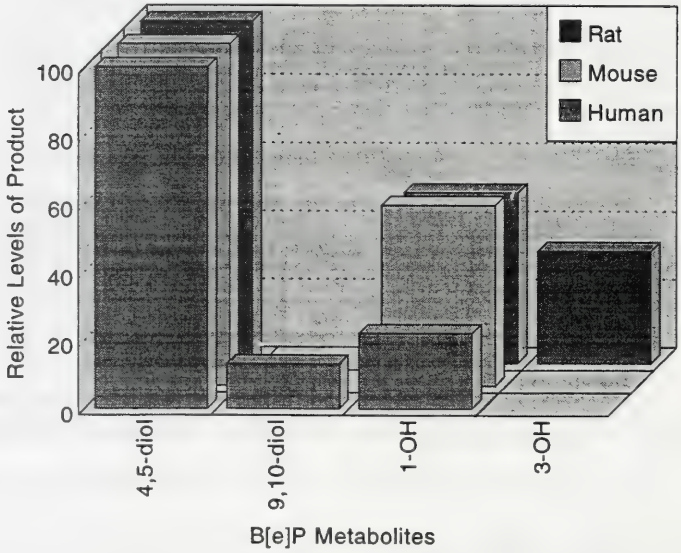
Jacob *et al.* did not report the initial amount of B[e]P used in the study. Therefore, metabolite levels in each species have been related to the metabolite found in the greatest quantity in each species. In each species the most abundant B[e]P metabolites formed was Benzo[e]pyrene, 4,5-dihydroxy-4,5-dihydro-, (±) (B[e]P-4,5-diol). The data in figure D.5.2-1 was derived from this relative data.

D.5.2.2.3 Discussion and Conclusions

The data contained in tables D.5.2-3 and D.5.2-4 and show that while the same metabolites are formed in the species studied, the levels of individual metabolites varies between species. The work of Selkirk *et al.* (JESL77) showed that for B[a]P, the major metabolite formed was the same in all three species. Jacob *et al.* (BJAC84) showed similar results for B[e]P metabolism. Although the major B[a]P metabolite was the same in the rat, mouse and hamster liver microsomes (B[a]P-3-hydroxy-) there are significant differences in the amounts of B[a]P-7,8-dihydroxy-7,8-dihydro-, (±) formed in all species. The formation of the B[a]P-7,8-diol is of particular interest as this compound serves as the precursor for Benzo[a]pyrene, 7,8-dihydroxy-9,10-epoxide-7,8,9,10-tetrahydro-, (±) (B[a]P-7,8-diol-9,10-epoxide), which is the active precursor which binds to DNA (see Section D.1 and Section D.4.2). The formation of

B[a]P-7,8-diol in the mouse is 1.8 fold greater than that in the rat and 2.7 fold greater than that in the hamster. Data from other appendices has shown that the mouse is more sensitive to the tumorigenic effects of PAH than either the rat or the hamster (see Main Document). The data presented by Selkirk *et al.* did not provide data on the levels of the individual stereoisomers of B[a]P-7,8-diol formed. This type of information is of particular concern as the conversion of B[a]P-7,8-diol to the ultimate diol-epoxide is dependent upon the configuration of the staring dihydrodiol (JLEV82). Only the (-) B[a]P-7,8-diol is converted to Benzo[a]pyrene, 7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro- (+) (CAS # 63323-31-9) ((+)-BDPE-II) which is the most active B[a]P-diol-epoxide (see Section D.4.2). Without knowing what differences in the (+) B[a]P-7,8-diol/(-) B[a]P-7,8-diol ratio exist between species, it is difficult to correlate differences in metabolite profiles with differences in species sensitivity to PAH.

Figure D.5.2-1: B[e]P Metabolite Profiles in Rat, Mouse and Human (BJAC84)



The metabolite profile for B[e]P show that in the rat and the mouse, the B[e]P-diol-epoxide precursor B[e]P-9,10-dihydroxy-9,10-dihydro, does not appear to be formed. The lack of formation of the diol-epoxide precursor may contribute to the low tumorigenic potency reported for B[e]P (see Main Document).

The shortage of adequate data makes it difficult to fully address the question outlined above. The available data suggests that metabolite profiles differ from species to species. However, without data which includes profiles for stereoisomers, it is difficult to correlate metabolite profiles with sensitivity to PAH. Species which produce more diol-epoxide precursors could be expected to be more sensitive to PAH tumorigenicity than species which produce only very low levels of diol-epoxide precursors. Further data is required before the validity of this assumption can be properly evaluated.

D.5.2.3 Are PAH-DNA Adduct Profiles The Same In Different Species?

This section examines the PAH-DNA adduct profiles in various species to determine if different in sensitivity to PAH tumorigenicity can be correlated with differences in the types of adducts found in various species.

D.5.2.3.1 Available Data

The majority of the available PAH-DNA adduct profile data in the literature report adduct profiles in various mouse tissues. Adduct profile data in other species is limited. Two studies which examined PAH-DNA adduct profiles in mice and rats were judged to have adequate data (JBAE81, JROJ86). Other studies which reported adduct profiles in single tissues or single species were not judged suitable for inclusion in the current data set. Administration and sacrifice protocols common to more than a single study could not be found. Therefore the data set could not be expanded beyond the two identified studies. These two studies used a common experimental protocol which should help to minimize experimental variation. Consequently, variations in adduct profiles can be attributed to species differences with a greater degree of confidence than if the profiles had been reported in different studies. For these reasons, it was felt that these two studies (JBAE81, JROJ86) represented the best data set. The data from these two studies are discussed below.

D.5.2.3.2 Results

Baer-Dubowska *et al.* examined B[a]P-DNA adduct formation in whole skin of female Swiss mice and male Wistar rats following topical application of B[a]P (JBAE81). Four major adducts were identified in both species (see table D.5.2-5). The majority of the adducts identified were derived from diol-epoxide metabolites of B[a]P. A minor amount of adducts remained unidentified. The identified adducts were Benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (-) ((-)BPDE-II) bound to deoxyguanosine (dG) ((-)-BPDE-II-dG); Benzo[a]pyrene, 7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+) ((+)-BPDE-II) bound to dG ((+)-BPDE-II-dG); Benzo[a]pyrene, 7α,8ß-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (±) ((±)-BPDE-I) bound to dG ((+)-BPDE-I-dG) and (+)-BPDE-II bound to deoxyadenosine (dA) ((+)-BPDE-II-dA). The major adduct was reported to be a mixture of (+)-BPDE-II-dG ans (+)-BPDE-I-dG. The authors suggest that the (±)-BPDE-I-dG represents a minor component of this peak.

Rojas *et al.* (JROJ86) reported B[a]P-DNA adducts profiles in male Swiss mice and male Wistar rats following topical application of B[a]P (see table D.5.2-6). This study provides a more detailed analysis than that provided by Baer-Dubowska *et al.*, in that the adducts which were unidentified by Baer-Dubowska *et al.* have been partially identified as Benzo[a]pyrene, 9-hydroxy (B[a]P-9-OH) bound to dG (B[a]P-9-OH-dG) and Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-, (±) (B[a]P-4,5-epoxy) bound to DNA (B[a]P-4,5-epoxy-DNA). The base attachment for this latter adduct was not identified.

Table D.5.2-5: **B[a]P-DNA Adduct Profiles in Mouse and Rat Skin Tissue (JBAE81).**

	% of Total Adducts	
Adduct	**Rat**	**Mouse**
Unknown	2.8	6.8
(-)-BPDE-II-dG	5.8	4.5
(+)-BPDE-II-dG (±)-BPDE-I-dG	87.1	84.6
(+) BPDE-I-dA	4.2	3.9

Table D.5.2-6: **B[a]P-DNA Adduct Profiles in Mouse and Rat Epidermal Tissue (JROJ86).**

	% of Total Adducts	
Adduct	**Rat**	**Mouse**
B[a]P-9-OH-dG	11.0	8.0
(-)-BPDE-II-dG	2.4	2.7
(+)-BPDE-II-dG (±)-BPDE-I-dG	81.7	84.9
(+) BPDE-I-dA	2.2	4.4
B[a]p-4,5-epoxy-DNA	2.7	-

D.5.2.3.3 Discussion and Conclusions

The data in table D.5.2-5 and D.5.2-6 show that adduct profiles in both the rat and mouse are essentially the same. In both species the major adduct would appear to be (+)-BPDE-II-dG. In both studies (JBAE81, JROJ86), the major adduct peak was reported as a mixture of (+)-BPDE-II-dG and (±)-BPDE-II-dG, however Baer-Dubowska *et al.* indicated that (±)-BPDE-I-dG represented only a minor component of this adduct peak (JBAE81). The binding data presented by Rojas *et al.* shows that the major adduct peak represents 84.9 percent of the total adduct in the mouse and 81.7 percent in the rat. The agreement between the two studies suggest that binding in epidermal tissue and whole skin are very similar in both species. In addition, it suggests that the binding profiles in skin in both species is essentially the same.

From the data presented it can be said that the PAH-DNA adduct profiles are the same in mouse and rat skin following topical application. The agreement between the two studies lends a reasonable degree of confidence to this observation. There is insufficient data to determine if the same degree of agreement would be obtained if the PAH were administered by another route. This type of information is critical in order to fully address the question outlined above.

With the current available data it would appear that PAH-DNA profiles are the same in the different species. However, the lack of data for routes other than topical application means that the overall confidence in this conclusion must be low.

D.5.3 Routes of Administration

D.5.3.1 Does The Route Of Administration Affect Tissue PAH Metabolite Profiles?

Data from other appendices have suggested that the route of administration can have a marked effect on the tumorigenic potency of PAH in a given tissue (see Main Document). One possible explanation for this

difference may lie in differences in PAH metabolite profiles found in a given tissue when PAH are administered via different routes. In order to determine the validity of this assumption, data which reported PAH metabolite profiles in multiple tissue were examined. The results are discussed below.

D.5.3.1.1 Available Data

There exists only limited data on B[a]P metabolite profiles in lung, liver and kidney following inhalation (Mitchell) and intratracheal administration (Weyand and Bevan). One study by Mitchell looked at total B[a]P in various tissues following inhalation, gavage and pelt application. Unfortunately, the data were not useable, primarily because the time between treatment and monitoring (24 hrs) was too long to detect B[a]P or its metabolites in any tissue except the gut, following oral administration.

Other work on *in vivo* metabolism is available. However, the majority of the data looks at metabolite levels in urine and feces and does not investigate metabolite profiles in individual tissues.

As a result of this lack of data it will not be possible to answer this question fully. However, examination of the available inhalation and intratracheal administration data does provide some useful information. The following section presents and discusses this information.

D.5.3.1.2 Results and Conclusions

The administration of B[a]P either by inhalation of by intratracheal administration results in a rapid distribution of B[a]P throughout the body (JMIT83, JWEY86). The work of Weyand and Bevan (JWEY86) provides the most detailed investigation of B[a]P absorption and tissue distribution (see figure D.5.3-1). As can be seen from figure D.5.3-1, intratracheal administration of B[a]P results in a rapid distribution of B[a]P and its metabolites throughout the body. The data presented in figure D.5.3-1 is a subset of the data provided by Weyand and Bevan (JWEY86). Data are also available for intestines, stomach, testis, spleen, heart, thymus, urine and blood.

Within 5 minutes of administration, B[a]P and its metabolites can be detected in the liver, kidney and blood as well as in the lung. It should be noted that the tissue levels were reported as percent of the total administered dose and were based on the levels of radioactivity detected in each tissue. Therefore these measures do not distinguish between B[a]P and B[a]P metabolites. Consequently, it must be assumed that both B[a]P and B[a]P metabolites are present. In the first 5 minutes after treatment, total B[a]P levels in the lung had dropped to approximately 60 percent of the administered dose. By ten minutes post treatment B[a]P levels in the liver had reached a maximum (see Figure D.5.3-1), and levels in the lung had dropped to approximately 42 percent of the total dose. At one hour post treatment, levels in the lung, liver and intestinal contents were essentially the same at 15 percent of the administered dose. As the length of time between treatment and measurement increases, the levels of B[a]P and metabolites in gut contents increases while levels in most tissues decrease. Mitchell et al. (JMIT83) reported similar results in Fisher 344 rats exposed to B[a]P by nasal inhalation. A direct comparison between the two studies is not possible as the total dose administered by Mitchell et al. is not reported.

Weyand and Bevan have also provided detailed metabolite profiles for lung and liver tissue 5, 60 and 360 minutes post administration (see Table D.5.3-1). The results in table D.5.3-1 are presented as percent of administered dose. By 360 minutes, it can be seen that there is little difference between the metabolite profiles in either tissue. The largest differences lie in the levels of unmetabolized B[a]P and 3-hydroxyB[a]P, both of which are higher in the lung. As the authors point out, it is difficult to determine if the metabolites found in a given tissue were produced in that tissue or if they presence results from transportation from another site. Therefore it is advantageous to monitor metabolite profiles as soon after treatment as is possible. For this reason, further discussion will focus on the metabolite profiles identified 5 minutes post treatment.

Figure D.5.3-1: *In Vivo* Fate in ^{3}H-B[a]P in Rats After Intratracheal Administration

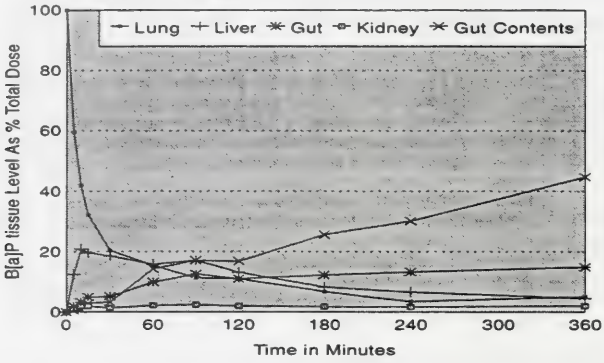


Table D.5.3-1: Tissue levels of B[a]P and B[a]P Metabolites in Rat Lung and Liver, 5, 60 and 360 After Intratracheal Administration. (Data from JWEY86)

	Liver (% Administered Dose)			Lung (% Administered Dose)		
Metabolite	5 Min	60 Min	360 Min	5 Min	60 Min	360 Min
B[a]P	8.61	4.72	0.28	32.15	5.18	0.43
3-Hydroxy	0.11	0.12	0.05	0.55	0.21	0.10
9-Hydroxy	0.08	0.12	0.05	0.43	0.21	0.05
4,5-Diol	0.10	0.17	0.06	0.42	0.12	0.06
7,8-Diol	0.11	0.11	0.05	0.53	0.17	0.07
9,10-Diol	0.15	0.38	0.12	0.87	0.29	0.10
1,6-Dione	0.27	0.25	0.10	1.91	0.22	0.06
3,6-Dione	0.23	0.32	0.13	2.40	0.48	0.09
6,12-Dione	0.25	0.38	0.13	1.28	0.39	0.05
Conjugates	0.32	1.09	0.42	2.02	1.38	0.35
Total	10.23	7.66	1.39	42.56	8.65	1.36

Five minutes post treatment, approximately 32 percent of the administered dose remains in the lung as unmetabolized B[a]P (see table D.5.3-1). Between 8 and 9 percent of the administered dose was identified in the liver as unmetabolized B[a]P. The combined total of unmetabolized B[a]P in the liver and lung represents approximately 53 percent of the administered dose. The remaining 47 percent presumably, will be distributed throughout the body and in the urine and feces as conjugates.

If liver and lung metabolism were the same, it would be reasonable to expect that the ratio of B[a]P to B[a]P metabolites would similar in both tissues. From the data in table D.5.3-2 it can be seen that the B[a]P metabolite/B[a]P profiles are very similar in both tissue. In general, B[a]P metabolites/B[a]P ratios in the lung are marginally higher than those reported in the liver. It is difficult to determine if these differences represent real differences in metabolism or if the increased levels in the lung represent the movement of metabolites from the liver to the lung. The greatest difference seems to be in the formation of B[a]P-3,6-dione which is 2.8 fold greater in the lung than in the liver. From this data however, it is not possible to determine if this represents a difference in cytochrome P-450 production of the B[a]P-3,6-dione, or if it represents a difference in the UDP-GT conjugation rate of the dione. Differences in production or removal, or differences in both production and conjugation could all produce the results seen in table D.5.3-2.

Table D.5.3-2: Ratios of B[a]P and B[a]P metabolites in Rat Liver and Lung 5 Minutes Post Treatment. (Data from JWEY86)

	% Unmetabolized B[a]P		
Metabolite	**Liver**	**Lung**	**Lung/Liver**
3-Hydroxy	1.3	1.7	1.3
9-Hydroxy	0.9	1.3	1.4
4,5-Diol	1.2	1.3	1.1
7,8-Diol	1.3	1.6	1.2
9,10-Diol	1.7	2.7	1.6
1,6-Dione	3.1	5.9	1.9
3,6-Dione	207	7.5	2.8
6,12-Dione	2.9	4.0	1.4
Conjugates	3.7	6.3	1.7

From the data presented above, it would seem that metabolism of PAH in both lung and liver are the same. The same products appear to be formed in approximately the same ratios. Unfortunately, studies with this level of detail are not available for other routes of administration. Therefore, it is not possible to address the initial question fully. However, two things appear to be clear

1) Liver and lung metabolism of PAH appear to be similar. There are some differences in the levels of certain PAH metabolites, but it is not possible to attribute these differences to any specific metabolic mechanism.

2) The same metabolites would appear to be formed in both tissues. It should however be noted that in both cases, the identified metabolites account for approximately 90 percent of the organic phase. Therefore, 10 percent of the organic phase remains unidentified. It is possible that the two tissues differ in the content of this unidentified segment of the organic phase. As the organic phase of the analysis appears to contain all of the primary B[a]P metabolites, it is possible that important metabolites are contained in the unidentified segment.

The lack of adequate metabolite profile data from other routes of administration makes it impossible to determine if administration of B[a]P by another route would result in differing tissue profiles in either the lung or the liver.

D.5.3.2 Do Differences in Enzyme Profiles Exist Between Tissues In A Single Species?

In order to fully address the objective outlined above, it is necessary to consider two questions.

i) Do levels of a given enzyme differ from tissue to tissue?

ii) Are the relative levels between Phase I and Phase II enzymes the same in different tissue?

It is important to understand the tissue distribution of each enzyme in order to estimate the levels of metabolites which may be formed. For example; if the levels of all Phase I and Phase II enzymes are the same in two tissue, it would be reasonable to expect that the levels of metabolites formed would be similar. It is not enough, however, to consider each enzyme in isolation. The ratios between Phase I and Phase II within a single tissue must also be considered. For example; if the levels of Phase I and Phase II enzymes a given tissue are 10 percent of those found in liver, and if the ratios between Phase I and Phase II enzymes are the same in each tissue, it is likely that the metabolite profiles in the two tissues would be similar. If however, the two tissue have differing Phase I and Phase II enzyme ratios, one would expect differing metabolite profiles in the two tissues. It is these differences in enzyme ratios and metabolite profiles which may, in part, be responsible for differing tissue sensitivities to the tumorigenic actions of PAH. For example; if the ratio between cytochrome P-450c and the other Phase I and Phase II enzymes in a given tissue is such that the formation of reactive metabolites is greater than that found in other tissues, it may be reasonable to expect that this tissue would show an increased sensitivity to PAH compared to other tissues. For these reasons, it is important to answer both of the questions outlined above. The following sections address each of these questions.

D.5.3.2.1 Available Data

Tissue profile data are available for Aryl Hydrocarbon Hydroxylase (AHH), Epoxide Hydrolase (EH) in the liver and a number of extra-hepatic tissue in the rat. Limited data are also available for the mouse. Tissue levels data in the rat provide the most complete data set (see Section D.3.2 and D.3.3). Therefore, only rat data was used in the subsequent analysis.

Unfortunately, data for the Phase II enzymes Glutathione-S-Transferase (GST), UDP-Glucuronyl Transferase (UDP-GT) and Sulphotransferase (ST) are extremely limited. The only data judged to be adequate provided GST levels in rat liver, lung and kidney.

Although tissue profiles data for all Phase I and Phase II enzymes are limited, there is sufficient information to address the questions outline above.

D.5.3.2.2 Tissue Profiles for Phase I and Phase II Enzymes

The information used to develop tissue profiles for cytochrome P-450c (AHH), E.H., GST, UDP-GT and ST are presented and discussed in detail in Section D.3. The following is intended as a summary of the wok presented in Section D.3. It must be stressed that the shortage of adequate data means that the level of confidence must be low. All of the tissue profiles presented herein would benefit from larger data sets which would help to confirm or refute the conclusion presented.

D.5.3.2.2.1 Enzyme Tissue Profiles in the Adult Rat

For all tissue profiles presented, the levels in extra-hepatic tissue have been compared to the levels in the liver. The numbers in parenthesis represent the percent of hepatic enzyme activity found in each tissue.

Relative Total Cytochrome P-450 Concentrations (pmoles P-450/mg microsomal protein):

Liver (100)	>>	Intestine (11)	>	Kidney (9.2)	>	Testis (6.4)	≈	Lung (5.9)

Relative AHH Activity:

Liver (100)	>>	Lung (8.9)	>	Kidney (5.1)	>	Testis (2.4)	≈	Skin (2.4)	>	Intestine (0.2)

Relative E.H. Activity:

Liver (100)	>>	Lung (8.9)	>	Kidney (5.1)	>	Testis (2.4)	≈	Skin (2.4)	>	Intestine (0.2)

Relative GST Activity:

Liver (100)	>>	Kidney (15.8)	>	Lung (5.1)

The tissue profiles presented above show that the levels of Phase I and Phase II enzymes are usually highest in the liver. Levels in most extra-hepatic tissues are generally less than 16 percent of those found in the liver. The only exception to this is in the testis. In the testis, the level of E.H. activity is 22 percent of that found in hepatic tissue. In the mouse, E.H. levels in the testis have been shown to be 2.4 fold greater than those in the mouse liver (see Section D.3.3.3).

While Phase I and Phase II enzymes activities are highest in the liver, the tissue profiles for each enzyme examined differ. Therefore it would be reasonable to assume that the metabolite profiles would differ between tissues. This difference may take one of two forms; either the actual products formed will be different or the levels of individual metabolites will be different between tissues. Data on PAH-DNA adduct formation shows that many of the same adduct are formed in many tissues (see Section D.4). Additionally, studies which have looked at B[a]P metabolite formation in rat lung and liver have shown that the same metabolites are formed in both tissues (JWEY86) (see Section D.5.3.1) This study did however, note differences in metabolite profiles between the two tissues. This may be an indication of differences in enzyme activities between the two tissues (see Section D.5.3.1). The following section examines the Phase I and Phase II enzyme profiles in individual tissues to establish if differences in the relative ratios of Phase I and Phase II enzymes do indeed exist.

D.5.3.2.2.2 Relative Enzyme Levels in Individual Tissues

The available AHH, E.H., and GST enzyme levels data are summarized in table D.5.3-3 through D.5.3-5. From these tables it can be seen that the largest data set is for AHH activity which has been monitored in numerous tissues. For both E.H. and GST, the data sets are smaller. Throughout this section, all enzyme activities are expressed as follows.

Activity = nmoles product formed/minute/mg of protein

D.5.3.2.2.2.1 AHH Levels in Rat Tissue.

Data on AHH levels are available in a number of tissues in a variety of rat strains including, Sprague-Dawley (S.D.), Wistar, Fisher 344 and Long-Evans. Additional data is available for neonatal S.D. rats. Although the neonatal data is included in table D.5.3-3 it has not been included in the consideration of adult AHH levels. This data was excluded because neonatal AHH levels are lower than adult levels and do not reach adult levels until 3 to 4 week postpartum (see Section D.2.1.1.4)

It can be seen from the data in table D.5.3-3 that there is moderate variation in hepatic AHH activity, ranging from 0.115 to 0.607 nmole/minute/mg protein, in the adult S.D. rat. The majority of the available data comes from S.D. rats. Single points are also available for Wistar, Fisher 344 and Long-Evans rats. The mean of hepatic AHH activity in the S.D. rat is 0.367±0.142. This agrees reasonably well with the activities reported for the other rat strains. Thus, it would seem that rat strain differences are minimal with respect to AHH activity. For this reason, it was felt that the data from all rat strains could be combined. The resultant AHH tissue levels for the "Adult Rat" are shown in table D.5.3-3. The data here, and throughout the remainder of the section are presented as Mean±Standard Error.

Table D.5.3-3: AHH Levels In Rat Hepatic and Extra-hepatic Tissue

	AHH Activity = nmoles product formed/min/mg microsomal protein								
Strain	Liver	Lung	Kidney	Whole Skin	Epidermis	Testis	Stomach	Intestine	Ref
S.D.	0.115		0.002			0.001	0.001		JSUZ80
S.D.	0.607	0.025	0.012			0.004		0.001	JGOL84
S.D.	0.379								JMCC87
Wistar	0.295	0.005	0.003						JGNO84
Fisher 344	0.313		0.006			0.002			JKLU81
Long-Evans	0.315								JAST88
?				0.004					JALU73
S.D. (N)	0.198			0.083					JBIC82
S.D. (N)	0.023			0.002					JASO85
S.D. (N)	0.002				0.001				JKHA89
S.D. (N)					0.001				JWAS89
S.D. Mean ±S.E.	0.37±0.14	0.013 ±0.012	0.005 ±0.002	0.004		0.003	0.001	0.001	
Adult Rat	0.34 0.14	0.01 0.007	0.005 0.002	0.004		0.002 0.001	0.001	0.001	

D.5.3.2.2.2.2 E.H. Levels in Rat Tissues

Data for EH levels are more limited than for AHH (see table D.5.3-4). The majority of the EH data comes from a single study by Oesch et al. (JOES77). In addition much of the presented data is derived from S. D. rats. The agreement between these studies is very good, showing little variation in reported activity. One study included in table D.5.3-4 which reported EH activity in Wistar rats, was excluded from further consideration as the assay protocol used in this report differed from that used in the others.

Table D.5.3-4: EH Levels In Rat Hepatic and Extra-hepatic Tissue

	EH Activity = nmoles product formed/min/mg microsomal protein								
Strain	Liver	Lung	Kidney	Whole Skin	Epidermis	Testis	Spleen	Intestine	Ref
S.D.	6.65±0.77[1]	0.33±0.05	0.75±0.01						JSCH78
S.D.	7.48±0.81			0.12±0.02					JBEN76
S.D.	6.1								JWAT87
S.D.	6.39±0.64	0.36 0.05	0.71 0.12		0.14 0.02	1.47 0.25	0.14 0.02	0.13 0.03	JOES77
Wistar	0.99 0.16	0.047 0.009	0.01 0.01						JGNO84
Mean S.E.	6.66 0.30	0.35 0.02	0.73 0.02	0.12	0.14	1.47	0.14	0.13	

1. Cited values are presented as mean standard deviation.
Calculated values are mean standard error.

D.5.3.2.2.2.3 GST Levels in Rat Tissue

Information about GST levels could only be located for liver, lung and kidney (See table D.5.3-5). As with both AHH and EH, the majority of the data that exists is derived from S.D rats. One study reports GST activity in Wistar rats (JGNO84). This latter study appears to have used the same substrate and assay protocol as that employed in measuring GST activity in S.D. rats. In addition, while the reported GST activity in Wistar rat was higher than that in the S.D. rats the variation in each study suggests that these differences are most likely to be due to experimental variation and not differences in strain. Therefore, data from both strains was combined to provide an "Adult Rat" mean and standard error value.

Table D.5.3-5: GST Levels In Rat Hepatic and Extra-hepatic Tissue

	GST Activity = nmoles product/min/mg protein			
Strain	Liver	Lung	Kidney	Ref
S.D.	475			JCHE88
S.D.	353 104[1]		199 19	JTAR91
S.D.	1054 138			JSTE87
Wistar	1171 138	59 9	186 23	JGNO84
Mean S.E.	763 204	59	193 7	

1. Cited values are presented as mean standard deviation.
Calculated values are mean standard error.

D.5.3.2.2.2.4 UDP-GT and ST Levels in Rat Tissue

There exists only limited data for both UDP-GT and ST levels in rat hepatic tissue and none in other tissues. Therefore, these enzymes could not be included in the consideration of tissue profiles.

D.5.3.2.3 Discussion

Phase I and Phase II enzyme profiles in rat liver, lung, kidney, testis and skin are summarized in table D.5.3-6 and in figures D.5.3-2 through D.5.3-5. In liver, lung and kidney, tissues for which AHH, EH and GST data are available, the basic enzyme profile is the same (see figures D.5.3-2 through D.5.3-5).

GST > EH > AHH

Table D.5.3-6: Phase I and Phase II Enzyme Profiles in Rat Tissue; Summary Table

	Enzyme Activity = nmoles product/min/mg protein		
Tissue	**AHH**	**EH**	**GST**
Liver	0.295 0.069	6.66 0.30	763 204
Lung	0.01 0.007	0.35 0.02	59
Kidney	0.005 0.002	0.73 0.02	193 7
Whole Skin	0.004	0.12	
Testis	0.002 0.001	1.47	
Stomach	0.001		
Intestine	0.001	0.13	
Epidermis		0.14	
Spleen		0.14	

Table D.5.3-7: GST Levels In Rat Hepatic and Extra-hepatic Tissue

	GST Activity = nmoles product/min/mg protein			
Tissue	**AHH**	**EH**	**GST**	**GST/EH**
Liver	1.00	23	2695	120
Lung	1.00	35	5900	169
Skin	1.00	30		
Kidney	1.00	146	38600	264
Testis	1.00	735		

Figure D.5.3-2: **Phase I and Phase II Enzyme Profiles In Rat Liver**

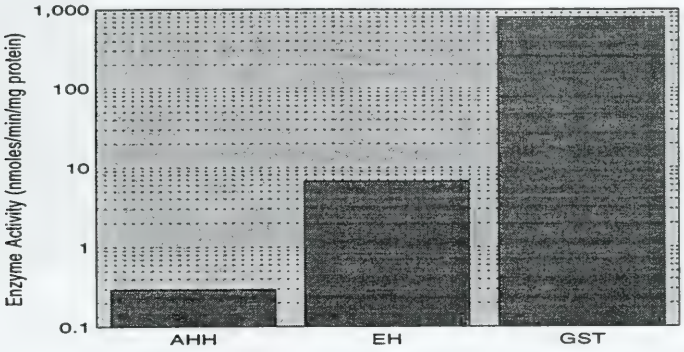


Figure D.5.3-3: **Phase I and Phase II Enzyme Profiles In Rat Lung**

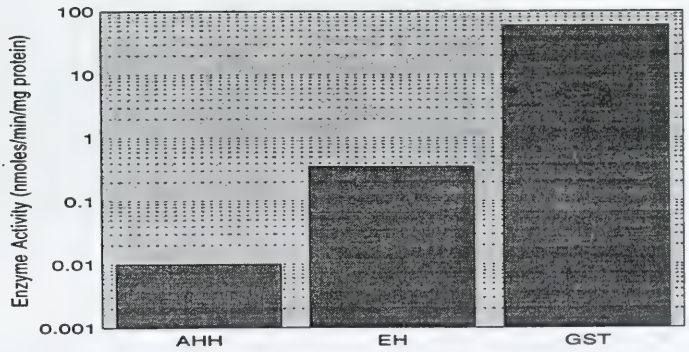


Figure D.5.3-4: Phase I and Phase II Enzyme Profiles In Rat Kidney

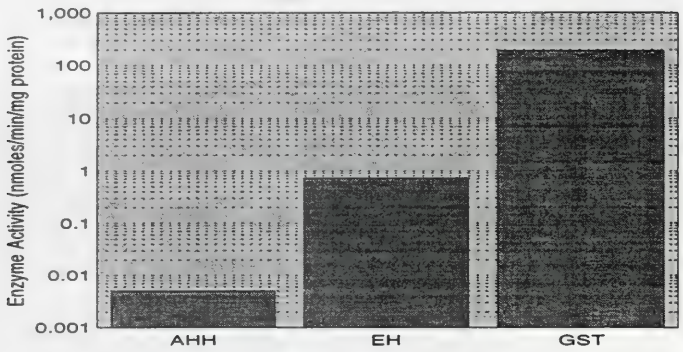
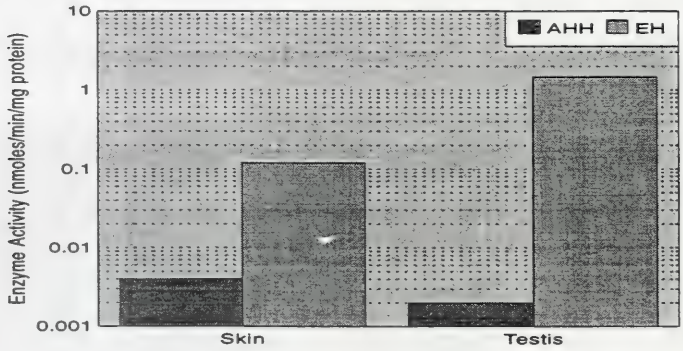


Figure D.5.3-5: Phase I Enzyme Profiles In Rat Testis and Skin



In liver and lung, the E.H./AHH ratios are similar, but there are differences in the GST/AHH ratios. This would suggest that metabolism of PAH in the two tissues would be similar; ie. the formation of reactive metabolites by AHH and the subsequent removal of these products by E.H. and GST would be similar in both tissues. As the levels of all Phase I and Phase II enzymes are lower in the lung than in the liver, it would be expected that the levels of product formed would also be lower. However, the metabolite profiles should be similar. In the kidney, the E.H./AHH and GST/AHH ratios are substantially larger than those in the liver or lung. This would suggest that the removal of AHH products would proceed more rapidly in the kidney than in other tissues. As a result, it might be expected that the metabolite profile in the kidney would be different from that seen in either the liver or the lung. It must be stressed that there is little data to support this hypotheses.

The data in table D.5.3-3 represent AHH tissue profiles from control animals. Induction of cytochrome P-450c activity using 3-MC produces some important changes which should be considered as many PAH are capable of inducing cytochrome P-450c (see Section D.2.1.1.4).

Table D.5.3-8 Induction of Rat Hepatic and Extra-hepatic AHH activity by 3-MC

	AHH Activity (pmoles/min/mg protein)			
Tissue	**Control**	**Induced**	**Fold Induction**	**Ref.**
Liver	0.04	0.856	21.5	JWIL90
Lung	0.00012	0.009	78.3	JWIL90
Kidney	0.00016	0.038	233	JWIL90
Epidermis	0.004	0.019	4.7	JVIZ80

The effects of induction with 3-MC on AHH, E.H. and GST activities have been discussed in detail in Section D.3. The results are summarized below. Treatment with 3-MC results in an increase in AHH activity in all tissues for which data is available (see table D.5.3-8). It had no appreciable effect on E.H. activity and only a moderate effect on GST activity (see Section D.3). While treatment with 3-MC increased AHH levels in all tissue, the largest increase were seen in lung and kidney. Without a concomitant increase in E.H. and GST levels, the increased capacity to produce reactive metabolites may be responsible, in part, for the increased tissue sensitivity seen in lung when compared to liver (See Main Document).

As discussed above, E.H./AHH and GST/AHH levels in liver and lung are similar in control animals. In treated animals however, the E.H./AHH and GST/AHH ratios in lung become smaller as the levels of AHH activity rise without an appreciable increase in either E.H. or GST activity. The result may be a reduced capacity for the lung to remove reactive PAH metabolites produced by AHH.

A 233 fold increase in kidney AHH levels was reported following treatment with 3-MC (JWIL90). Unlike the lung, the GST/AHH ration in the kidney is very large. Glutathione-S-transferase acts primarily on primary epoxides (see Sections D.1 and D.2). Therefore, the large excess of GST in the kidney should be able to conjugate and remove and additional epoxides produced by AHH as a result of the increase in AHH levels.

D.5.3.2.4 Conclusions

It is difficult to determine how these differences in enzyme profiles between tissues are related to differences in tissue sensitivity to PAH. The initial activation of PAH to the primary epoxide is carried out by AHH. Both EH and GST are able to use the epoxides formed by AHH as substrate for further metabolic processing (see Section D.2). The difference between the two enzymes lies in the products each produces. Epoxides conjugated by GST are effectively removed from further processing by AHH and therefore are unavailable for diol-epoxide formation. Epoxides subjected to EH action are converted to dihydrodiol. In some cases these dihydrodiols are the required precursors for diol-epoxide formation by AHH (see Section D.1).

The formation of diol-epoxides capable of binding to DNA is thought to be a crucial step in PAH carcinogenesis. Therefore tissues which favour the formation of dihydrodiols over the formation of GST conjugates are likely to be more sensitive to the tumorigenic effects of PAH than those in which the majority of the epoxides formed are conjugated and removed. Unfortunately it is not possible to draw any firm conclusions about the relationship between tissue enzyme profiles and sensitivity to PAH primarily because there is insufficient data. The data presented in table D.5.3-7 shows an interesting trend however, which is worthy of some further investigation. Liver, lung and skin all show EH/AHH ratios that range between 23 and 35 while kidney and testis have values of 146 and 735 respectively. In addition, the GST/AHH ratio in kidney is 38600. Both testis and kidney appear to be less sensitive to the tumorigenic effects of PAH than the other tissues cited above. It is conceivable that the greater levels of EH and in particular GST provides increased protection to these tissues by greatly reducing the amount of epoxide available for conversion to diol-epoxide. It must be stressed that this is largely conjecture and a great deal of missing information is needed before any real relationships can be identified. For example, based on

the data presented in table D.5.3-7 it would appear that liver should be the most sensitive tissue. This however, has been show to be incorrect. Consideration of the remaining Phase II enzymes, UDP-GT and ST, may provide further data that would explain the differences in sensitivity between liver , lung and skin. Until this information is available, it will not be possible to establish any creditable relationship.

D.5.3.3 Does The Route Of Administration Affect PAH-DNA Adduct Formation?

As the formation of PAH-DNA adducts is thought to be a critical step in the development of PAH induced tumors it is necessary to know if differences in the route of administration result in differences in the types of adducts formed in a given tissue. If different routes of administration result in different types of adducts being formed in a given tissue, this might help to explain the observed differences in PAH tumorigenic potency when administered by different routes (see Main Document). This sections summarizes the data presented in Section D.4, and addresses the question outlined above.

D.5.3.3.1 Available Data

There appears to be only limited data regarding DNA adduct formation in multiple tissues following, inhalation, oral or dermal administration of PAH. Total PAH-DNA binding data for both topical application and intratracheal installation is limited to a single study for each route (JHUG90) and (JWEY87) respectively. Total PAH-DNA adduct formation in multiple tissues following oral administration of PAH provides a slightly larger dataset. Two studies have examined DNA binding in female ICR mice (JLU*86) and A/HeJ mice (JSTO84).

A proper and complete analysis of the question identified above is hampered by a number of factors including;

i) Lack of data.

ii) Lack of a consistent dose between studies.

iii) Lack of a standard sacrifice time.

iv) Lack of a standardized format for expressing total PAH-DNA adduct formation.

As mentioned above, the number of studies which report the type of total PAH-DNA binding data required to address the present question is limited. In addition, the various studies which do report adequate data fail to use a common dose which could be used to directly compare data. In one instance (JLU*86), the reported dose was 200 µmoles/Kg, whereas other studies report the total amount administered. Therefore without knowing the weight of each mouse used in the former study, it is not possible to compare absolute levels of adduct formation between these studies. Hughes et al (JHUG90) reported adduct formation in skin and lung following topical application of PAH animals sacrificed 6 hours post treatment whereas most other studies sacrificed animals 24 hours post treatment. To further complicate comparisons, there is no standard format for reporting levels of adduct formation. The two most commonly used formats are the following.

i) pmoles adduct/ mg DNA

ii) nmoles adduct/mole DNA-Phosphate

This latter format is referred to as the Relative Adduct Level (RAL). Unfortunately, the two formats are not readily interchangeable. Thus direct comparison between the two data formats is not possible. For these reasons, it is not possible to compare tissue distribution of adduct formation on an absolute binding basis. It is possible, however, to compare the results on a relative basis. The available data is presented and discussed in the following sections.

D.5.3.3.2 Results

D.5.3.3.2.1 Total Binding: Tissue Profiles Following Topical Application

Total PAH-DNA binding data in multiple tissues following topical application of PAH is summarized in table D.5.3-9. Only one study was found to contain adequate data. Unfortunately data was restricted to skin and lung tissue. As can be seen from the data in table D.5.3-9, the level of total binding in lung is approximately 15.4 % of that reported in skin.

For each of the three routes of administration, total binding in multiple tissues are compared to adduct levels in the tissue which is first exposed to the PAH. Thus; for topical application, skin is the first tissue to contact PAH, and binding levels are set relative to total adduct formation in the skin. For oral administration, forestomach and intestine were chosen as the primary tissues as these would be the tissues of first contact in the two studies which reported oral data. For intratracheal installation, lung tissue was assumed to be the tissue of first contact.

Table D.5.3-9: Total PAH-DNA Binding Tissue Profiles: Topical Application

Species/Strain	Tissue	Total Binding[1]	Relative Binding	Ref
Mouse (Parkes)	Skin	7.8	100	JHUG90
Mouse (Parkes)	Lung	1.2	15.4	JHUG90

1) Total Binding in pmoles adduct/mg DNA

It should be noted that the total binding levels for skin and lung were taken from different times post-treatment. Total PAH-DNA binding data reached a maximum at 24 hours post-treatment, while in lung, maximal binding was not reached until 48 hours post-treatment. It would be preferable to compare adduct formation at a single time point (maximal in skin). However, the 24 hour binding data in lung tissue was not available. Therefore it was decided to compare maximal binding levels in both tissues even though this may alter the ratio.

D.5.3.3.2.2 Total Binding: Tissue Profiles Following Oral Administration

Total PAH-DNA binding data in multiple tissues following oral administration of PAH is summarized in table D.5.3-10. Two studies were identified which had adequate data. However, the work of Stowers et al. (JSTO84) does not report total binding, but rather reports the binding levels of a number of identified B[a]P-DNA adducts. These were combined to provide an estimate of the total binding. It is realized that there are some inherent problems associated with this in so far as total binding levels will be underestimated in most tissues. This is caused by the exclusion of a small number of unidentified adducts from the presented data. In studies which report both total PAH-DNA binding and adduct profile data, the unidentified adducts generally account for less than 10 percent of the total (see Section D.4.2). Therefore,, while the potential for errors exists, it was felt that the benefit of having a larger dataset, and the increased confidence this may provide, would outweigh the errors which might be introduced through its inclusion.

In both mouse strains tested, the highest levels of adduct formation are found in the primary tissue (see table D.5.3-10). Lung and liver show the highest levels of adduct formation in non-primary tissue. The remaining tissues for which data is available show lower levels of adduct formation. Direct comparison of the total binding data between the two studies is not possible for a number of reasons which are outlined below.

i) The studies use separate and incompatible formats for reporting adduct formation.
Lu et al use relative adduct levels (JLU*86).
Stowers et al. used pmoles adduct/mg DNA (JSTO84).

ii) Different primary tissues were used;
Lu et al. reported adduct levels in intestine (JLU*86).
Stowers et al. reported adduct levels in forestomach (JSTO84).

iii) Different sacrifice times were used.
Lu et al reported adduct levels 24hrs post-treatment (JLU*86).
Stowers et al reported adduct formation 48 hrs post-treatment (JSTO84).

Table D.5.3-10: Total PAH-DNA Binding Tissue Profiles: Oral Administration

Species/Strain	Tissue	Total Binding	Relative Binding	Ref
Mouse (A/HeJ)	Forestomach	0.058[1]	100	JSTO84
	Lung	0.04	69	JSTO84
	Liver	0.023	40	JSTO84
	Kidney	0.017	29	JSTO84
	Brain	0.016	28	JSTO84
	Colon	0.015	26	JSTO84
	Muscle	0.017	28	JSTO84
Mouse (ICR)	Intestine	13.0[2]	100	JLU*86
	Liver	4.9	38	JLU*86
	Lung	4.2	32	JLU*86
	Kidney	2.4	19	JLU*86
	Brain	1.2	9	JLU*86
	Heart	3.3	25	JLU*86
	Skin	1.9	15	JLU*86

1) Total Binding in pmoles adduct/mg DNA 2) Relative Adduct Levels

D.5.3.3.2.3 Total Binding: Tissue Profiles Following Intratracheal Installation

Total PAH-DNA binding data in multiple tissues following intratracheal installation of PAH is summarized in table D.5.3-11. Only one study was identified as containing adequate data. Unfortunately this study used male Sprague-Dawley (S.D.) rats, while the studies which report topical and oral data used mice. Therefore comparison between inhalation and other routes is hampered by species differences in addition to the problems identified above.

Table D.5.3-11: Total PAH-DNA Binding Tissue Profiles: Intratracheal Installation

Species/Strain	Tissue	Total Binding[1]	Relative Binding	Ref
Sprague-Dawley	Lung	0.013	100	JWEY86
Sprague-Dawley	Liver	0.033	254	JWEY86

1) Total Binding in pmoles adduct/mg DNA

As can be seen from table D.5.3-11 total adduct formation is higher in liver than in lung despite the fact that lung is the primary tissue. The reasons for this are unclear. Weyand et al. (JWEY86) showed that intratracheally instilled B[a]P was rapidly removed from lung tissue and distributed throughout the body. Levels in liver approached those in lung within 10 minutes post-treatment (see Section D.5.3.1). This rapid distribution may reduce the amount of PAH available for activation in lung tissue. Further investigation is required before any meaningful conclusions can be drawn from this data. It would appear however, that unlike topical and oral administration, intratracheal administration results in the highest levels of adduct formation at a site other than the primary tissue. Given that different species were used for the topical and oral and inhalation routes, it is difficult to determine if this is a real route difference or if it reflects species differences.

D.5.3.3.3 Discussion and Conclusions

For topical and oral administration the greatest level of adduct formation was found in the primary tissue. Intratracheal administration of PAH would appear to result in the highest level of adduct formation in liver and not in the primary tissue. However as mentioned above, it is not possible to determine if this difference reflects a route difference or a species difference.

Hughes et al. (JHUG90) examined both total binding and adduct profiles in skin and lung tissue following topical application of B[a]P. The results showed that while total binding was lower in the lung than in the skin, the adduct profiles in both tissue were essentially the same (see Section D.4.2). This may suggest that the route of administration does not affect the types of adduct which are formed. This is however a very preliminary conclusion and requires adduct profile data in lung tissue from other routes of administration to aid in its confirmation.

The main conclusion which can be drawn from this data is that the route of administration alters the level of adduct formation in any given tissue. However, the amount of data available and the problems associated with comparison between these data drastically reduce the confidence in the conclusion.

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APPENDIX E

STANDARDIZED NOMENCLATURE FOR POLYNUCLEAR AROMATIC HYDROCARBONS

by Bryan Leece

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APPENDIX E STANDARDIZED NOMENCLATURE FOR POLYNUCLEAR AROMATIC HYDROCARBONS

E.1 Introduction

Polynuclear aromatic hydrocarbons (PAH) have been the subject of scientific investigation for a considerable length of time. During this time, the nomenclature of the compounds has proceeded in a less than standard fashion. The result is a profusion of chemical names and synonyms. This can lead to a great deal of confusion when reviewing published data. Loening and Merritt have reviewed and identified some of the problems than can arise from the use of non-standardized PAH nomenclature (1). In addition, Loening and Merritt have provided an extremely useful list of PAH names and synonyms for classical and heterocyclic PAH. The list identifies the PAH names preferred under the nomenclature rules established by the International Union of Pure and Applied Chemistry (IUPAC). Unfortunately, the list does not extend to names and synonyms for PAH metabolites. It is in the naming of PAH metabolites that the confusion arising from the lack of a standardized nomenclature becomes particularly acute. Table E.1 provides a list of synonyms found in the literature that have all been used to identify a specific enantiomeric metabolite of benzo[a]pyrene. A standard nomenclature for PAH and their metabolites would, in large part, help to reduce the confusion caused by the use of multiple synonyms.

In order for a standardized system to gain wide acceptance, it must be as simple to use. It would be of benefit if it retained as many of the nomenclature features currently in use as possible, while adhering to the rules of nomenclature as established by IUPAC. The nomenclature approach outlined here, tries to achieve this objective, both for PAH parent compounds and the larger group of PAH metabolites.

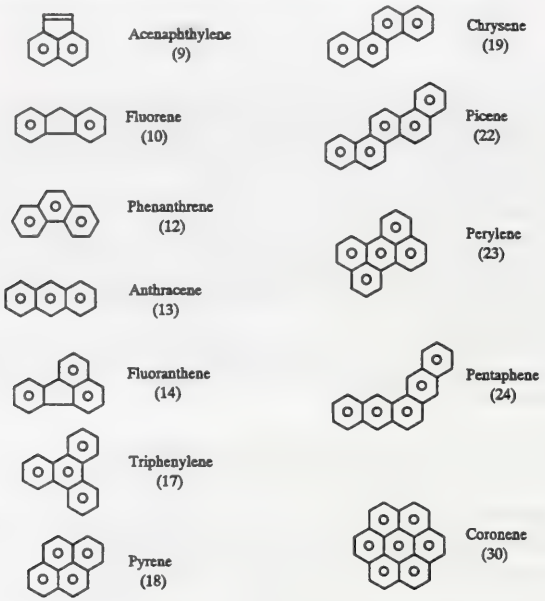
Table E.1: Synonym list for the benzo[a]pyrene diol-epoxide metabolite:
Benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, ±

± trans 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene
± trans 7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene
± 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene
benzo[a]pyrene-7,8-dihydrodiol-9,10-oxide, anti
Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide, anti
Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide, trans anti
Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, (7.alpha., 8.beta., 8a.beta.,9a.beta.), trans-(.+-.)
Benzo[a]pyrene-7ß,8α-diol-9α,10α-epoxide
Trans 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene

E.2 IUPAC Nomenclature For Classical PAH

The International Union of Pure and Applied Chemistry (IUPAC) has established a system for naming and numbering polynuclear aromatic hydrocarbons (PAH)(2). IUPAC Rule A-21 outlines the accepted uses of trivial and semi-trivial names for identifying fused polycyclic hydrocarbon systems. A number of trivial names have been retained to identify a core set of PAH. From this set, the names of other PAH compounds can be derived. The names, structures ans Chemical Abstract Registry (CAS) numbers are shown in figure E.1. A more complete list can be found under Rule A-21.1 in (2).

Figure E.1: IUPAC Accepted Trivial Names for Fused Polycyclic Hydrocarbons



Names for fused ring systems for which accepted trivial names do not exists are derived from combinations of accepted trivial names. Names for these compounds are created by combining names of compounds from the list of accepted trivial names. IUPAC Rules A-21.3 and A-21.4 outline the procedures and constraints for developing names for these compounds. In addition, Loening and Merritt have reviewed and simplified these procedures (1).

Benzo[a]pyrene, dibenz[a,h]anthracene and many other common PAH have names that are derived in this manner. For these compounds, the name of the core PAH is preceded by the accepted name of the addition. The location of the ring fusion is indicated by the letter or letters located in the square brackets.

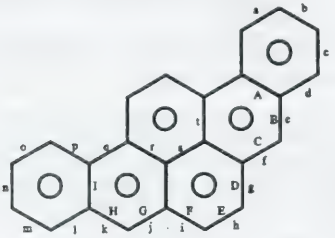
Although naming many polycyclic compounds in this manner is straight forward, there are some fairly common compounds for which more than one name would be acceptable by IUPAC definition. The examples in figures E.2A and E.2B illustrate this point. The compound in figure E.2A has two acceptable IUPAC names; dibenzo[a,i]pyrene and benzo[rst]pentaphene. The ring lettering for the pyrene core is shown in lower case while the lettering for the pentaphene core is shown in upper case. While either name is acceptable, benzo[rst]pentaphene is the preferred name because it uses the larger core name and requires a simpler prefix, benzo rather than dibenzo.

The compound in figure E.2B also has two IUPAC acceptable names; naphtho[1,2,3,4-def]chrysene and the more commonly cited dibenzo[a,e]pyrene. IUPAC Rule A-21.3 states that when naming PAH which do not have a IUPAC defined core or base name (2):

> "The base component should contain as many rings as possible, provided that it has a trivial name, and the name should occur as far as possible from the beginning of Rule A-21.1"

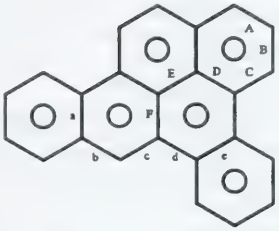
Figure E.2: Nomenclature For Non-core PAH Compounds

E.2A



Dibenzo[a,i]pyrene
Benzo[rst]pentaphene

E.2B



Dibenzo[a,e]pyrene
Naphtho[1,2,3,4-def]chrysene

In this case, pyrene occurs at position 18 and chrysene at position 19 in Rule A-21.1. However, Rule A-21.3 also states that the attached group should be as simple as possible. The rule goes on to provide the example shown in figure E.3. The acceptable name for the compound shown in figure E.3 is dibenzophenanthrene and not naphthophenanthrene, because, "benzo" is a simpler structure than "naphtho", even though there are two "benzo" rings and only one "naphtho" ring (2). In the case of the compound in figure E.2B, it is not immediately clear which component of Rule A-21.3 takes precedence over the other. If the choice of base component is the predominant factor, then naphtho[1,2,3,4-def]chrysene would be the appropriate name for this compound. If, however, the object in naming the compound is to keep the name as simple as possible, then using dibenzo[a,e]pyrene would be a better choice. The reasons for this are three fold. Firstly, the difference in distance from the beginning of Rule A-21.1 between pyrene and chrysene is only one position (18 and 19). The fusion structure required in using dibenzo[a,e]pyrene is considerably easier and more concise than that required for naphtho[1,2,3,4-def]chrysene. In addition, the final fusion site in dibenzo[a,e]pyrene is closer to the initial face of the base molecule (e) than the final fusion face in naphtho[1,2,3,4-def]chrysene (f). Secondly, conforming to the second item in Rule A-21.3, "benzo" is a simpler structure then "naphtho". Thirdly, the name dibenzo[a,e]pyrene is in much more common usage than naphtho[1,2,3,4-def]chrysene. While citing "common usage" is perhaps not the most scientific of arguments, it does suggest that it is a nomenclature format that most people understand and will utilize.

Figure E.3: IUPAC example of substituent name selection based on Rule A-21.3

Dibenzophenanthrene

E.3 Nomenclature For Substituted PAH and PAH Metabolites

The International Union of Pure and Applied Chemistry indicates that three types of nomenclature can be used to identify PAH and their metabolites; Additive, Fusion and Substitutive (2). The basis for these three approaches are outlined by Loening and Merritt (1). Additive nomenclature is discouraged by IUPAC and is not in common usage in the literature. Therefore, it will not be discussed here. The remaining two systems, are discused below.

E.3.1 Fusion Nomenclature:

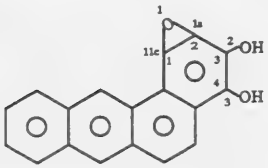
Fusion nomenclature is used by CAS in the database listing of PAH and PAH metabolites. It came into wide use by CAS in the ninth collective index (3). The use of this nomenclature has advantages and disadvantages. Both should be considered when deciding on a standardized nomenclature.

Fusion nomenclature is fully supported by IUPAC. It is a highly descriptive and very precise method of identifying PAH and metabolite compounds. This format odes not rely on the use of trivial names other than those sanctioned by IUPAC (Rule A-21.1). Further, the rigorous requirements of this system reduce the potential for errors in stoichiometry.

The disadvantages of fusion nomenclature lie in the names that are given to some PAH and PAH metabolites. As discussed above, the names for some parent PAH can be substantially different from those in common usage (dibenzo[a,e]pyrene and naphtho[1,2,3,4-def]chrysene). The largest alteration in compound name occurs when naming PAH epoxides and diol-epoxide metabolites. Consider the two compounds in figure E.4. Both are epoxide metabolites of benz[a]anthracene. But to those unfamiliar with fusion nomenclature, this may not be immediately apparent, if the chemical structures are not provided. In addition, the inclusion of the oxirene ring as part of the cyclic core results in a renumbering of the parent PAH core. This can be extremely confusing.

Figure E.4: Substitutive and Fusion Nomenclature for two metabolites of benzo[a]pyrene

E.4A



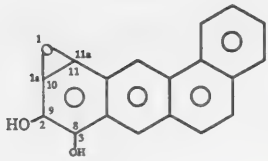
Substitutive:

Benz[a]anthracene, 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydro, (±)

Fusion:

Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-,

E.4B



Substitutive:

Benz[a]anthracene, 8,9-dihydroxy-10,11-epoxy-8,9,10,11-tetrahydro-, (±)

Fusion:

benz[7,8]anthra[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-,

E.3.2

E.3.3 Substitutive Nomenclature:

Substitutive nomenclature of PAH is governed by IUPAC Rules C-15.1 through C-16.36 (2). Variations of this nomenclature format are uses extensively in the PAH literature. As with fusion nomenclature, substitutive nomenclature has both advantages and disadvantages that must be weighed when developing a standardized nomenclature.

The advantages of the substitutive system lie in its ease of use. Because this approach allows the use of common or trivial root names for all parent PAH and metabolites, it is easier to relate a wide range of metabolites back to their parent compounds. This is of particular concern for epoxide and diol-epoxide metabolites. Furthermore, this method maintains the original ring numbering from parent to daughter compounds.

There are a number of disadvantages associated with the substitutive approach to PAH nomenclature. Trivial names for parent compounds that are not included in IUPAC Rule A-21.1 are constructed using fusion nomenclature. Therefore, the use of substitutive nomenclature to identify the metabolites of these compounds results in a mixed nomenclature format. Further, substitutive nomenclature may not be as precise as fusion nomenclature. In addition, there is a greater chance for stoichiometric error when naming compounds with this approach (1).

Despite the problems that may be associated with the use of substitutive nomenclature, the ease with which it can be used by a wide range of people and its prevalence in the literature suggest that it may be a more easily accepted approach to naming PAH and their metabolites. For example, using substitutive nomenclature, the name for B[a]P-7,8-diol-9,10-epoxide would be:

7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene

rather than the fusion name of

benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-

The synonym list in appendix E1 uses a modification of the substitutive approach which is equally valid. The names will appear with the root name first followed by the substituent groups. Therefore, the names will be presented in the following format:

benzo[a]pyrene, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-

The advantage of this is that it allows the reader to tell at a glance what the root or parent compound is. Following each entry will be a list of synonyms that have been found in the literature.

Some discussion may be raised regarding the alphabetic ordering of this name. Strict adherence to substitutive nomenclature as outlined by Loening and Merrit (1) would place the epoxy group before the dihydroxy. In this case the di prefix modifies the hydroxyl term. Because the hydroxyl is the modified functional group, it should rightly be place after the epoxy group in strict alphabetical order. However, this approach cites IUPAC Rule C-16.0 in general and C-16.33 specifically. Further examination of IUPAC rule C-16.34 indicates that the name of a prefix or substituted substituent is considered to begin with the first letter of its proper name. Thus, a dihydroxy substituent name is considered to start with the letter "d" rather than the "h" of the hydroxyl group. Applying IUPAC Rule C-16.34 to the PAH situation supports the name arrangement proposed here.

E.3.4 Nomenclature for PAH Substituents

For the majority of substituted PAH and PAH metabolites, nomenclature does not present a problem. Names are formed simply by identifying the substituent and its location on the parent compound. For example a benzo[a]pyrene that has an hydroxyl groups attached to carbon 1 is usually referred to 1-hydroxybenzo[a]pyrene with little variation in the literature. However, some PAH metabolites are given a range of names in the literature. For example, epoxide metabolites can be referred to as epoxide, epoxy, oxide or oxirene, and a dihydrodiol can be identified as a dihydrodiol, diols, dihydroxy or as a dihydroxy-dihydro-. The use of multiple names can lead to confusion and misinterpretation of data if the compounds are incorrectly identified. The following sections attempt to address this problem for these and other PAH derivatives where multiple names exist.

E.3.4.1 Naming Epoxides:

Throughout the PAH literature, the terms "oxide", "epoxy" and "epoxide" are used interchangeably to describe the three membered ring structure formed from an oxygen bridge between two adjacent carbon atoms on the PAH core.

IUPAC rule C-33.1 supports the use of the term "oxide" to describe this structure when radicofunctional nomenclature is being used to describe the entire molecule (1). However, PAH nomenclature does not usually use this nomenclature form (1,2). Furthermore, the term "oxide" can also be used to describe anions formed by the loss of a proton from an alcohol or a phenol or ethers of the form R^1-O-R^2 (IUPAC Rules C-84.2 and C-211.1 respectively) (2). For these reasons, the use of the term "oxide" to describe the three membered C-O-C ring structure should be discouraged in PAH nomenclature.

IUPAC does not seem to distinguish between the terms "epoxide" and "epoxy", although the latter seems to be the preferred form. IUPAC rules B-15.1 and B-15.2 describe and oxygen bridge between two carbons of an aromatic ring system as an "epoxy" group (2). The formation of the epoxy group is not restricted to two adjacent carbons in the PAH core. IUPAC rule 212.2 states; that if the term "epoxy" is used in the name of an aromatic compound, it must be accompanied by the appropriate "dihydro" designation (2).

PAH-epoxides and diol-epoxides should be names according to the IUPAC suggested rules for naming epoxy compounds. IUPAC Rule C-16.11 defines the term "epoxy" as detachable. Therefore, the synonym list in appendix E1 will use the format given below to identify all PAH epoxides and diol-epoxides.

Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-

E.3.4.2 Naming Cyclic Aldehydes:

Numerous methods for naming PAH containing aldehyde substituents can be found in the literature. For example; a -CHO group can be identified as either a formyl- or a carbaldehyde. IUPAC Rule C-304.1 (2) states:

> *The name of an aldehyde in which the aldehyde is attached directly to the carbon atom of the ring system is formed by adding the suffix "carbaldehyde", "di-carbaldehyde", etc, to the name of the ring system.*

Thus: 7-formylbenz[a]anthracene, would be identified as "benz[a]anthracene, 7-carbaldehyde"

E.3.4.3 Naming Aromatic Ketones:

Naming PAH compounds that contain a single aromatic ketone does not present a problem. In almost all cases these compounds are identified with the suffix "one". For example benzo[a]pyrene-6-one. Problems arise when naming compounds that have two or more keto groups. In the literature, these are referred to as either quinones of diones. IUPAC Rule C-317.1 (2) indicates that the use of the terms "quinone" and "di-quinone" is acceptable for aromatic compounds in which two or four CH groups are converted to C=O groups, providing that the necessary rearrangement of double bonds is required to provide the quinone structure and maintain aromaticity occurs. Further, the terms "quinone" or "diquinone" are added as a suffix to the name of the parent compound (2). In appendix E1, names for these compounds will be presented using the following format:

Benzo[a]pyrene, 3,6-quinone

E.4 Stereochemical Identification of PAH Metabolites:

Stereochemical configuration is an important determinant in conferring biological activity upon a molecule. PAH metabolites are no exception. The absolute configuration of hydroxyl and epoxide groups of a PAH diol-epoxide ultimately determines the ability of the metabolite to bind to DNA. For this reason, it is very important to be able to distinguish between different diol-epoxide diastereomers and enantiomers when evaluating their respective biological activities. For example: benzo[a]pyrene, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro is a generic name which characterizes 8 individual and distinct compounds that differ in their arrangement of hydroxy and epoxide groups around the 7,8,9,10-ring. Two schemes are currently in use in the literature to identify the absolute configuration around these carbon atoms of a diol-epoxide. One method of describing absolute configuration has borrowed the α and ß notation from steroid nomenclature. The other method uses the Cahn-Ingold-Prelog Sequence Rule "R" and "S" notation to describe the absolute configuration around each individual carbon.

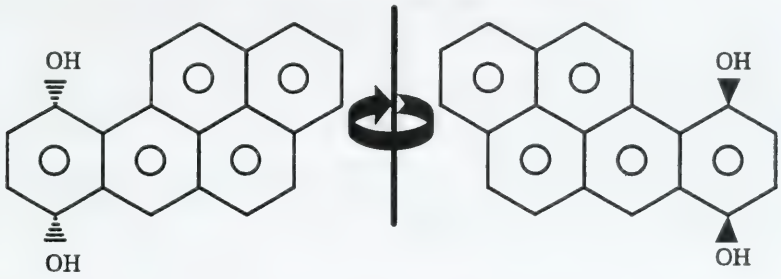
The "R" and "S" notation has an advantage in that it does not require any further assumptions about the orientation of the molecule. That is, one does not need the molecule to be oriented in any specific way for the "R" and "S" designation to remain in effect. If this approach has a disadvantage, it is that it does not present an immediate picture of the molecule to persons unfamiliar with stereochemical notation. Further, visualization often requires the use of three dimensional models.

The use of α and ß notation draws on steroid nomenclature and more readily presents a picture of the molecule than the "R" and "S" notation. It does however have certain drawbacks which must be identified. The use of α and ß imposes restrictions on the way a molecule is presented and on the ways in which it can be moved through space when examining the configuration. IUPAC Rule E-4.11 suggests that steroid nomenclature can be applied to other compounds if the absolute configuration is known (2). IUPAC Rule F-6.2 (2) further states:

"When the rings of a quasi-planar parent structure are denoted as projections onto the plane of the paper, an atom or group which lies below the plane of the paper is denoted α, and an atom or group which lies above the plane are denoted β. Furthermore, whenever this system is used, the orientation depicted for the parent structure MUST be maintained."

The reason for this requirement for consistency becomes clear if one considers a planar molecule with groups projecting above and below the molecular plane. Those groups located above the plane will be identified as ß and those below as α (see Fig E.5). If one now rotates the molecule through the plane of the paper, groups previously found projecting above the plane will now be found below the plane and those originally found below the plane will be above (see Fig. E.5.)

Figure E.5: Use of α and ß as Stereochemical Descriptors in PAH Nomenclature



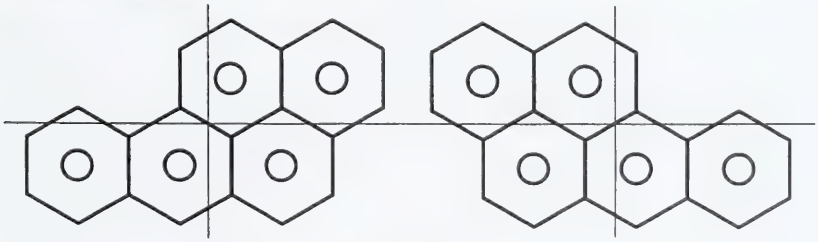
Benzo[a]pyrene, 7α,10α-dihydroxy- Benzo[a]pyrene, 7ß,10ß-dihydroxy-

To prevent this miss-assignment some rules must be used to insure the correct orientation of the PAH core molecule. IUPAC Rule 1e (2) states:

The polycyclic system is oriented so that the greatest number of rings are in a horizontal row and a maximum number of rings are above and to the right of the horizontal (upper right quadrant) (see Fig. 6)

In the synonym list in appendix E1, the α and ß notation will be used to describe the stereochemical configuration of PAH metabolites because it is more readily understood by a wider range of readers. Further, PAH ring structure orientation will follow that outlines by IUPAC in Rule 1e. It is understood that there are potential hazards associated with the adoption of this approach, but it is felt that the advantages outweigh these drawbacks.

Figure E.6: IUPAC Orientation of PAH Molecules



E.5 Conclusions:

There is no definitive way to name PAH and their metabolites. Even within IUPAC guidelines, there is more than one acceptable approach to PAH nomenclature. Unfortunately, this has lead to a profusion of names for some of the PAH metabolites. The problem is greatest when naming PAH diol and diol-epoxide metabolites. As these are the compounds of greatest biological interest because of their association with the biological activity of PAH, this multiplicity of names can hamper inter-lab comparisons of data. This paper and the attached synonym appendix are presented in an attempt to provide some clarity to the present situation.

In suggesting the use of substitutive nomenclature rather than fusion nomenclature, a number of things were taken into consideration. Substitutive nomenclature, in one form or another, is used by the majority of the people who publish in this field. Therefore, it is the format that most people are familiar with. Maintaining both parent name and ring numbering makes it easier to identify the specific metabolites of concern and reduces the likelihood of errors is substituent location. However, use of substituent nomenclature may increase the risk of incorrect stoichiometry. The use of α and ß notation from steroid nomenclature provides an absolute configuration for each metabolite where this would be a concern. Using this format does, however, restrict the movement of the molecule in space. At present, in most publications where structures are provided, the IUPAC rules of molecule orientation are observed. This reduces the concern over molecular orientation.

The only area where there has been a deviation from IUPAC convention is in the naming of hydroxy and dihydroxy compounds. IUPAC suggests that these compounds should be labeled as "-ols" and "-diols". In the synonym list in appendix E1, this has been replaced by "hydroxy" and "dihydroxy" because it is felt that this format is less ambiguous than the format suggested by IUPAC.

The synonym list provides not only compound names but CAS registry numbers for those compounds where CAS number exist. A great deal of confusion could be alleviated if future publications were to provide CAS numbers in conjunction with compound names. This would allow a much easier cross-comparison of data, but would not require the adoption of yet another nomenclature format for PAH.

References:

1) Loening, K., Merrit, J. Later, D., Wright, W.: Polynuclear Aromatic Hydrocarbons: Nomenclature Guide; Battelle Press, Columbus, Ohio, U.S.A., 1990

2) International Union of Pure and Applied Chemistry, Organic Chemistry Division; Nomenclature of Organic Chemistry; Sections A,B,C,D,E,F and H; Pergamon Press, Oxford England, 1979.

3) American Chemical Society Chemical Abstract Service, Chemical Registry File, 1991

Appendix E1

The following list of PAH synonyms has been compiled from names cited in the literature. It is not intended to be a complete list, but rather, it focuses on PAH dihydrodiols and diol-epoxides as this is the area where the largest number of synonyms exist. Each citation provides the name of the compound, based on the nomenclature approach suggested above, the CAS registry name and number and a number of cited synonyms. In addition, where appropriate, the Cahn-Ingold-Prelog Sequence notation for absolute configuration has been provided as well as the standard α and ß notation.

A note of explanation with regard to the CAS registry stereochemical notation of diol-epoxides may also prove useful. In identifying the stereochemical configuration of a diol-epoxide, CAS fixes the configuration around at one of the four carbons and provided a relative configuration around the remaining three. For example, for the compound shown in figure E.4A, one configuration would be:

Benz[a]anthracene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-

The CAS name for this is:

Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro,
[1aS-(1a.alpha.,2.beta.,3.alpha.,11c.alpha.)]

Here, the absolute stereochemistry at carbon 1a is fixed as "S" and the stereochemistry around the remaining carbons is given relative to this. It should also be noted that the relative locations around carbons 1a and 11c refer to the positions of the hydrogens and not the epoxide, while the relative configurations around carbons 2 and 3 refer to the position of the hydroxyl groups. Loening and Merritt have provided a detailed description of this format in the Polynuclear Aromatic Hydrocarbon Nomenclature Guide (1).

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E1.1 Benzacridines

E1.1.1 Benz[a]acridine 3,4-diol-1,2-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]acridine, 3α,4β-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydro-, (±)	85993-46-0	
Synonyms: Oxireno[5,6]benz[1,2-a]acridine-2,3-diol, 1a,2,3,11c-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,11c.alpha.)- Benz[a]acridine, 3β,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±) (±) Trans-3α,4β-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydrobenz[a]acridine		
Benz[a]acridine, 3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	117019-74-6	
Synonyms: Oxireno[5,6]benz[1,2-a]acridine-2,3-diol, 1a,2,3,11c-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,11c.alpha.)-(.+-.)- Benz[a]acridine, 3β,4α-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydro-, (±) (±) Trans-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz[a]acridine		

E1.1.2 Benz[c]acridine 3,4-diol-1,2-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benz[c]acridine, 3α,4β-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydro-, (±)	78215-29-9	
Synonyms: Oxireno[3,4]benz[1,2-c]acridine-2,3-diol, 1a,2,3,11c-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,11c.alpha.)-(.+-.)- Benz[c]acridine, 3α,4β-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydro-, (±) (±) Trans-3α,4β-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydrobenz[c]acridine		
Benz[c]acridine, 3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	78167-99-4	
Synonyms: Oxireno[3,4]benz[1,2-c]acridine-2,3-diol, 1a,2,3,11c-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,11c.alpha.)-(.+-.)- Benz[c]acridine, 3β,4α-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydro-, (±) (±)-trans-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz[c]acridine		

E1.2 Benz[a]anthracene

E1.2.1 Benz[a]anthracene Diols

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 1,2-dihydroxy-1,2-dihydro-, trans-, (±)	64807-77-8	
Synonyms: Benz[a]anthracene-1,2-diol, 1,2-dihydro-, trans-(.+-.) Benz[a]anthracene, 1α,2β-dihydroxy-1,2-dihydro-, (±) Benz[a]anthracene-1,2-dihydrodiol		
Benz[a]anthracene, 1,2-dihydroxy-1,2-dihydro-8-(hydroxymethyl)-, trans-	73195-10-5	
Synonyms: Benz[a]anthracene-1,2-diol, 1,2-dihydro-8-(hydroxymethyl)-, trans- Trans-1,2-dihydroxy-1,2-dihydro-8-hydroxymethylbenz[a]anthracene		
Benz[a]anthracene, 3β,4α-dihydroxy-3,4-dihydro-, (+)	67335-43-7	3S,4S
Synonyms: Benz[a]anthracene-3,4-diol, 3,4-dihydro-, (3S-trans) (+)-Benz[a]anthracene-3,4-dihydrodiol		
Benz[a]anthracene, 3α,4β-dihydroxy-3,4-dihydro-, (-)	67335-42-6	3R,4R
Synonyms: Benz[a]anthracene-3,4-diol, 3,4-dihydro-, (3R-trans) (-)-Benz[a]anthracene-3,4-dihydrodiol		
Benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-, trans-, (±)	64501-86-6	
Synonyms: Benz[a]anthracene-3,4-diol, 3,4-dihydro-, trans-(.+-.) Benz[a]anthracene, 3α,4β-dihydroxy-3,4-dihydro-, (±) Benz[a]anthracene, 3β,4α-dihydroxy-3,4-dihydro-, (±) Trans-3,4-dihydroxy-3,4-dihydrobenz[a]anthracene (±)-trans-3,4-dihydroxy-3,4-dihydrobenz[a]anthracene Benz[a]anthracene-3,4-dihydrodiol		
Benz[a]anthracene, 3,4-dihydroxy-1,2,3,4-tetrahydro-, trans-, (±)	80399-21-9	
Synonyms: Benz[a]anthracene-3,4-diol, 1,2,3,4-tetrahydro-, trans-(.+-.) Benz[a]anthracene, 3α,4β-dihydroxy-1,2,3,4-tetrahydro-, (±) Benz[a]anthracene, 3β,4α-dihydroxy-1,2,3,4-tetrahydro-, (±) Trans-3,4-dihydroxy-1,2,3,4-tetrahydrobenz[a]anthracene		
Benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-7-methyl-, trans-, (±)	74634-54-1	
Synonyms: Benz[a]anthracene-3,4-diol, 3,4-dihydro-7-methyl-,trans-, (.+-.) (±)-trans-3,4-dihydroxy-3,4-dihydro-7-methyl-Benz[a]anthracene		

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-8-methyl-, trans-	94849-77-1	
Synonyms: Benz[a]anthracene-3,4-diol, 3,4-dihydro-8-methyl-, trans- Trans-3,4-dihydroxy-3,4-dihydro-8-methylbenz[a]anthracene		
Benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-8-(hydroxymethyl)-, trans-	78617-05-7	
Synonyms: Benz[a]anthracene-3,4-diol, 3,4-dihydro-8-(hydroxymethyl)- Trans-3,4-dihydroxy-3,4-dihydro-8-hydroxymethylbenz[a]anthracene		
Benz[a]anthracene, 5,6-dihydroxy-5,6-dihydro-, trans-, (±)	67315-17-7	
Synonyms: Benz[a]anthracene-5,6-diol, 5,6-dihydro-, trans-(.+-.) Benz[a]anthracene, 5α,6β-dihydroxy-5,6-dihydro-, (±) Benz[a]anthracene, 5β,6α-dihydroxy-5,6-dihydro-, (±) Benz[a]anthracene-5,6-dihydrodiol		
Benz[a]anthracene, 5,6-dihydroxy-5,6-dihydro-8-methyl-, trans-, (±)	94850-06-3	
Synonyms: Benz[a]anthracene-5,6-diol, 5,6-dihydro-8-methyl-, trans-, (.+-.) Trans-5,6-dihydroxy-5,6-dihydro-8-methylbenz[a]anthracene		
Benz[a]anthracene, 5,6-dihydroxy-5,6-dihydro-8-(hydroxymethyl)-,trans-	77102-53-5	
Synonyms: Benz[a]anthracene-5,6-diol, 5,6-dihydro-8-(hydroxymethyl)-, trans- Trans-5,6-dihydroxy-5,6-dihydro-8-hydroxymethylbenz[a]anthracene		
Benz[a]anthracene, 8,9-dihydroxy-8,9-dihydro-, trans-, (±)	67262-34-4	
Synonyms: Benz[a]anthracene-8,9-diol, 8,9-dihydro-, trans-(.+-.) Benz[a]anthracene, 8α,9β-dihydroxy-8,9-dihydro-, (±) Benz[a]anthracene, 8β,9α-dihydroxy-8,9-dihydro-, (±) Benz[a]anthracene-8,9-dihydrodiol		
Benz[a]anthracene, 8,9-dihydroxy-8,9-dihydro-8-methyl-, trans-	94849-87-1	
Synonyms: Benz[a]anthracene-8,9-diol, 8,9-dihydro-8-methyl-, trans- Trans-8,9-dihydroxy-8,9-dihydro-8-methylbenz[a]anthracene		
Benz[a]anthracene, 10,11-dihydroxy-10,11-dihydro-, (±)	60967-90-0	
Synonyms: Benz[a]anthracene-10,11-diol, 10,11-dihydro-, trans-(.+-.) Benz[a]anthracene, 10α,11β-dihydroxy-10,11-dihydro- (±) Benz[a]anthracene, 10β,11α-dihydroxy-10,11-dihydro-, (±) Benz[a]anthracene-10,11-dihydrodiol		

E1.2.2 Benz[a]anthracene 3,4-diol-1,2-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydro-, (±)	64551-89-9	
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-, 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene Benz[a]anthracene-3,4-diol-1,2-epoxide		
Benz[a]anthracene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-,	80433-79-0	1R,2S,3R,4S
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-, [1aS-(1a.alpha.,2.alpha.,3.beta.,11c.alpha.)]- Trans-3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene		
Benz[a]anthracene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-,	80433-78-9	1S,2R,3S,4R
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-, [1aR-(1a.alpha.,2.alpha.,3.beta.,11c.alpha.)]- Trans-3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene		
Benz[a]anthracene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±)	64598-81-8	
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,11c.alpha.)-(.+-.) Benz[a]anthracene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±) (±)-trans-3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene Benz[a]anthracene-3,4-diol-1,2-epoxide 1		
Benz[a]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-,	80433-81-4	1S,2R,3R,4S
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-, [1aR-(1a.alpha.,2.beta.,3.alpha.,11a.alpha.)]- Trans-3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene		
Benz[a]anthracene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-,	80466-23-7	1R,2S,3S,4R
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-, [1aS-(1a.alpha.,2.beta.,3.alpha.,11a.alpha.)]- Trans-3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene		
Benz[a]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	64598-80-7	
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,11c.alpha.)-(.+-.) Benz[a]anthracene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±) (±)-trans-3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene Benz[a]anthracene-3,4-diol-1,2-epoxide 2		
Benz[a]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-7-methyl-1,2,3,4-tetrahydro-, trans-, (±)	64625-66-7	
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-1,2-diol,1a,2,3,11c-tetrahydro-6-methyl-,(1a.alpha.,2.beta.,3.alpha.,11c.alpha.)-, 7-methyl-Benz[a]anthracene-3,4-diol-1,2-epoxide 7-MBenz[a]anthracene-trans-3,4-diol-anti-1,2-epoxide		

E1.2.3 Benz[a]anthracene 1,2-diol-3,4-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-, (±)	064530-45-6	
Synonyms: Benzo[6,7]phenanthro[1,2-b]oxirene-10,11-diol, 1a,10,11,11a-tetrahydro-, Benz[a]anthracene-1,2-diol-3,4-epoxide		
Benz[a]anthracene, 1α,2B-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (±)	64869-93-8	
Synonyms: Benzo[6,7]phenanthro[1,2-b]oxirene-10,11-diol, 1a,10,11,11a-tetrahydro-, (1a.alpha.,10.beta.,11.alpha.,11a.alpha.)-(.+-.)- Benz[a]anthracene, 1B,2α-dihydroxy-3B,4B-epoxy-1,2,3,4-tetrahydro-, (±) Benz[a]anthracene-1,2-diol-3,4-epoxide 1		
Benz[a]anthracene, 1α,2B-dihydroxy-3B,4B-epoxy-1,2,3,4-tetrahydro-, (±)	64838-75-1	
Synonyms: Benzo[6,7]phenanthro[1,2-b]oxirene-10,11-diol, 1a,10,11,11a-tetrahydro-, (1a.alpha.,10.alpha.,11.beta.,11a.alpha.)-(.+-.)- Benz[a]anthracene, 1B,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (±) Benz[a]anthracene-1,2-diol-3,4-epoxide 2		

E1.2.4 Benz[a]anthracene 10,11-diol-8,9-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 10,11-dihydroxy-8,9-epoxy-8,9,10,11-tetrahydro-,	064550-80-7	
Synonyms: Benzo[5,6]phenanthro[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, Benz[a]anthracene-10,11-diol-8,9-epoxide		
Benz[a]anthracene, 10α,11B-dihydroxy-8B,9B-epoxy-8,9,10,11-tetrahydro-, (±)	64598-85-2	
Synonyms: Benzo[5,6]phenanthro[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, Benz[a]anthracene, 10B,11α-dihydroxy-8α,9α-epoxy-8,9,10,11-tetrahydro-, (±) Benz[a]anthracene-10,11-diol-8,9-epoxide 1		
Benz[a]anthracene, 10α,11B-dihydroxy-8α,9α-epoxy-8,9,10,11-tetrahydro-, (±)	64598-84-1	
Synonyms: Benzo[5,6]phenanthro[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, Benz[a]anthracene, 10B,11α-dihydroxy-8B,9B-epoxy-8,9,10,11-tetrahydro-, (±) Benz[a]anthracene-10,11-diol-8,9-epoxide 2		

E1.2.5 Benz[a]anthracene 8,9-diol-10,11-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 8,9-dihydroxy-10,11-epoxy-8,9,10,11-tetrahydro-, (±)	53760-21-7	
Synonyms: Benz[7,8]anthra[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, Benz[a]anthracene-8,9-diol-10,11-epoxide 8,9-dihydrodiol-10,11-epoxy-benz[a]anthracene Benz[a]anthracene-8,9-diol-10,11-epoxide 1		
Benz[a]anthracene, 8ß,9α-dihydroxy-10ß,11ß-epoxy-8,9,10,11-tetrahydro-,	64937-39-9	8R,9S,10R,11S
Synonyms: Benz[7,8]anthra[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, [1aR-(1a.alpha.,2.alpha.,3.beta.,11b.alpha.]- Trans-8ß,9α-dihydroxy-10ß,11ß-epoxy-8,9,10,11-tetrahydrobenz[a]anthracene		
Benz[a]anthracene, 8α,9ß-dihydroxy-10α,11α-epoxy-8,9,10,11-tetrahydro-, (±)	64598-82-9	
Synonyms: Benz[7,8]anthra[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,11b.alpha.)-(.+-.)- Benz[a]anthracene, 8ß,9α-dihydroxy-10ß,11ß-epoxy-8,9,10,11-tetrahydro-, (±) (±)-trans-8ß,9α-dihydroxy-10ß,11ß-epoxy-8,9,10,11-tetrahydrobenz[a]anthracene Benz[a]anthracene-8,9-diol-10,11-epoxide 1		
Benz[a]anthracene, 8ß,9α-dihydroxy-10α,11α-epoxy-8,9,10,11-tetrahydro-,	64937-40-2	8R,9S,10S,11R
Synonyms: Benz[7,8]anthra[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, [1aS-(1a.alpha.,2.beta.,3.alpha.,11b.alpha.)]- Trans-8ß,9α-dihydroxy-10α,11α-epoxy-8,9,10,11-tetrahydrobenz[a]anthracene		
Benz[a]anthracene, 8α,9ß-dihydroxy-10ß,11ß-epoxy-8,9,10,11-tetrahydro-, (±)	64598-83-0	
Synonyms: Benz[7,8]anthra[1,2-b]oxirene-2,3-diol, 1a,2,3,11b-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,11b.alpha.)-(.+-.) Benz[a]anthracene, 8ß,9α-dihydroxy-10α,11α-epoxy-8,9,10,11-tetrahydro-, (±) (±)-trans-8ß,9α-dihydroxy-10α,11α-epoxy-8,9,10,11-tetrahydrobenz[a]anthracene Benz[a]anthracene-8,9-diol-10,11-epoxide 2		

E1.2.6 Benz[a]anthracene 7,12-Dimethyl-: Carbaldehyde Derivatives

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 7-carbaldehyde-12-methyl-,	013345-61-4	
Synonyms: Benz[a]anthracene-7-carboxaldehyde, 12-methyl- 7-formyl-12-methylbenz[a]anthracene		
Benz[a]anthracene, 12-carbaldehyde-7-methyl-,	017513-40-5	
Synonyms: Benz[a]anthracene-12-carboxaldehyde, 7-methyl- 12-formyl-7-methylbenz[a]anthracene		
Benz[a]anthracene, 7,12-dicarbaldehyde-,	019926-22-8	
Synonyms: Benz[a]anthracene-7-12-dicarboxaldehyde 7,12-diformylbenz[a]anthracene		

E1.2.7 Benz[a]anthracene 7,12-Dimethyl-: Diol-Epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-,	111001-46-8	1R,2S,3S,4R
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-6,11-dimethyl-, [1aS-(1a.alpha.,2.beta.,3.alpha.11c.alpha.)]-		
Benz[a]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-,	111001-45-7	1S,2R,3R,4S
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-6,11-dimethyl-, [1aR-(1a.alpha.,2.beta.,3.alpha.,11c.alpha.)]-		
Benz[a]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)	130856-45-0	
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-6,11-dimethyl-, (1a.alpha.,2.beta.,3.alha.,11c.alpha.)-(.+-.)- Benz[a]anthracene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)		
Benz[a]anthracene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)	115225-74-6	
Synonyms: Benzo[6,7]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11c-tetrahydro-6,11-dimethyl-, (1a.alpha.,2.alpha.,3.beta.,11c.alpha.)-(.+-.)- Benz[a]anthracene, 3ß,4α-dihydroxy-1α,2α-epoxy-7,12-dimethyl-1,2,3,4-tetrahydro-, (±)		

E1.2.8 Benz[a]anthracene 7,12-Dimethyl-: Diol Derivatives

Compound/Synonyms	CAS #	Absolute Configuration
Benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-7,12-dimethyl-, trans-, (±)	74938-86-6	
Synonyms: Benz[a]anthracene-3,4-diol, 3,4-dihydro-7,12-dimethyl-, trans-(.+-.) Benz[a]anthracene, 3α,4β-dihydroxy-3,4-dihydro-7,12-dimethyl-, (±) Benz[a]anthracene, 3β,4α-dihydroxy-3,4-dihydro-7,12-dimethyl-, (±) Trans-3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz[a]anthracene Trans-3,4-dihydro-3,4-dihydroxy-7,12-dimethylbenz[a]anthracene 7,12-dimethylbenz[a]anthracene-3,4-dihydrodiol 7,12-dimethylbenz[a]anthracene-3,4-diol Trans-3,4-dihydroxy-3,4-dihydro-DMBA DMBA-3,4-diol		
Benz[a]anthracene, 8,9-dihydroxy-8,9-dihydro-7,12-dimethyl-, trans-, (±)	74938-87-7	
Synonyms: Benz[a]anthracene-8,9-diol, 8,9-dihydro-7,12-dimethyl-, trans Benz[a]anthracene, 8α,9β-dhydroxy-8,9-dihydro-7,12-dimethyl-, (±) Benz[a]anthracene, 8β,9α-dihydroxy-8,9-dihydro-7,12-dimethyl-, (±) Trans-8,9-dihydroxy-8,9-dihydro-7,12-dimethylbenz[a]anthracene Trans-8,9-dihydroxy-8,9-dihydro-DMBA DMBA-8,9-diol		
Benz[a]anthracene, 5,6-dihydroxy-5,6-dihydro-7,12-dimethyl-, trans-, (±)	75262-88-3	
Synonyms: Benz[a]anthracene-5,6-diol, 5,6-dihydro-7,12-dimethyl-, trans-, Benz[a]anthracene, 5α,6β-dihydroxy-5,6-dihydro-7,12-dimethyl-, (±) Benz[a]anthracene, 5β,6α-dihydroxy-5,6-dihydro-7,12-dimethyl-, (±) Trans-5,6-dihydroxy-5,6-dihydro-7,12-dimethylbenz[a]anthracene Trans-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene 7,12-dimethylbenz[a]anthracene-5,6-dihydrodiol Trans-5,6-dihydroxy-5,6-dihydro-DMBA DMBA-5,6-dihydrodiol		
Benz[a]anthracene, 10,11-dihydroxy-10,11-dihydro-7,12-dimethyl, trans-, (±)	92622-79-2	
Synonyms: Benz[a]anthracene-10,11-diol, 10,11-dihydro-7,12-dimethyl-, trans-(.+-.) Benz[a]anthracene, 10α,11β-dihydroxy-10,11-dihydro-7,12-dimethyl-, (±) Benz[a]anthracene, 10β,11α-dihydroxy-10,11-dihydro-7,12-dimethyl-, (±) Trans-10,11-dihydroxy-10,11-dihydro-7,12-dimethylbenz[a]anthracene Trans-10,11-dihydro-10,11-dihydroxy-7,12-dimethylbenz[a]anthracene		
Benz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-7,12-dimethyl-, trans-, (±)		
Synonyms: Benz[a]anthracene, 3α,4β-dihydroxy-3,4-dihydro-7,12-dimethyl-, (±) Benz[a]anthracene, 3β,4α-dihydroxy-3,4-dihydro-7,12-dimethyl-, (±) 7,12-dimethylbenz[a]anthracene-3,4-dihydrodiol 7,12-dimethylbenz[a]anthracene-3,4-diol		

E1.3.1 Benzo[a]pyrene Diols

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[a]pyrene, 4,5-dihydroxy-4,5-dihydro-, trans-, (±)	50700-50-0	
Synonyms: Benzo[a]pyrene-4,5-diol, 4,5-dihydro-, trans-(.+-.) Benzo[a]pyrene, 4α,5β-dihydroxy-4,5-dihydro-, (±) Benzo[a]pyrene, 4β,5α-dihydroxy-4,5-dihydro-, (±) Trans-4,5-dihydroxy-4,5-dihydrobenzo[a]pyrene Trans-4,5-dihydroxybenzo[a]pyrene Benzo[a]pyrene-4,5-dihydrodiol		
Benzo[a]pyrene, 7α,8β-dihydroxy-7,8-dihydro-, (+)	62314-67-4	
Synonyms: Benzo[a]pyrene-7,8-diol, 7,8-dihydro-, (7S-trans) (+)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (+)-Benzo[a]pyrene-7,8-dihydrodiol		
Benzo[a]pyrene, 7β,8α-dihydroxy-7,8-dihydro, (-)	60864-95-1	
Synonyms: Benzo[a]pyrene-7,8-diol, 7,8-dihydro-, (7R-trans) (-)-trans-7β,8α-dihydroxy-7,8-dihydrobenzo[a]pyrene (-)Benzo[a]pyrene-7,8-dihydrodiol		
Benzo[a]pyrene, 7,8-dihydroxy-7,8-dihydro-, trans-, (±)	61443-57-0	
Synonyms: Benzo[a]pyrene-7,8-diol, 7,8-dihydro-, trans-(.+-.) Benzo[a]pyrene, 7α,8β-dihydroxy-7,8-dihydro-, (±) (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene, trans Trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene Benzo[a]pyrene-7,8-dihydrodiol B[a]P-7,8-dihydrodiol Benzo[a]pyrene-7,8-diol		
Benzo[a]pyrene, 7,8-dihydroxy-7,8,9,10-tetrahydro-, trans-, (±)	64314-00-7	
Synonyms: Benzo[a]pyrene-7,8-diol, 7,8,9,10-tetrahydro-, trans-(.+-.) Benzo[a]pyrene, 7α,8β-dihydroxy-7,8,9,10-tetrahydro-, (±) (±)-trans-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene		

Compound/Synonyms		CAS #	Absolute Configuration
Benzo[a]pyrene, 9,10-dihydroxy-9,10-dihydro-, trans-, (±)		58030-91-4	
Synonyms:	Benzo[a]pyrene-9,10-diol, 9,10-dihydro-, trans-(.+-.) Benzo[a]pyrene, 9α,10ß-dihydroxy-9,10-dihydro-, (±) Benzo[a]pyrene, 9ß,10α-dihydroxy-9,10-dihydro-, (±) Trans-9,10-dihydroxy-9,10-dihydrobenzo[a]pyrene 9,10-dihydro-dihydroxybenzo[a]pyrene (trans) Benzo[a]pyrene-9,10-dihydrodiol		
Benzo[a]pyrene, 7,8-dihydroxy-7,8-dihydro-9-methyl-, trans-, (±)		73998-81-9	
Synonyms:	Benzo[a]pyrene-7,8-diol, 7,8-dihydro-9-methyl-, trans- Benzo[a]pyrene, 7α,8ß-dihydroxy-7,8-dihydro-9-methyl-, (±) Benzo[a]pyrene, 7ß,8α-dihydroxy-7,8-dihydro-9-methyl-, (±) Trans-7,8-dihydroxy-9-methyl-7,8-dihydrobenzo[a]pyrene		

E1.3.2 Benzo[a]pyrene 7,8-diol-9,10-epoxides

Compound/Synonyms		CAS #	Absolute Configuration
Benzo[a]pyrene, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-, (±)		55097-80-8	
Synonyms:	Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, (±)-trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Benzo[a]pyrene-7,8-diol-9,10-epoxide Benzo[a]pyrene-7,8-diol-9,10-epoxide		
Benzo[a]pyrene, 7α,8ß-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (±)		63323-29-5	7S,8R,9S,10R
Synonyms:	Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, [7S-(7.alpha.,8.beta.,8a.beta.,9a..beta.)]- (+)-Benzo[a]pyrene-7α,8ß-diol-9α,10α-epoxide 1 (+) Benzo[a]pyrene-7,8-diol-9,10-epoxide 1		
Benzo[a]pyrene, 7ß,8α-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (-)		63357-09-5	7R,8S,9R,10S
Synonyms:	Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, [7R-(7.alpha.,8.beta.,8a.beta.,9a.beta.)]- (-)-7ß,8α-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (-)-7ß,8α-diol-9ß,10ß-epoxide 1 (-)-Benzo[a]pyrene-7,8-diol-9,10-epoxide 1		

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[a]pyrene, 7α,8ß,-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (±)	58917-91-2	
Synonyms: Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, (7.alpha.,8.beta.,8a.beta.,9a.beta.)-(.+-.) (±)-7ß,8α-dihydroxy-9ß,10ß-7,8,9,10-tetrahydrobenzo[a]pyrene (±)-trans-7ß,8α-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Benzo[a]pyrene, 7ß,8α-dihydroxy-9ß,10ß-7,8,9,10-tetrahydro-, (±) Benzo[a]pyrene-7ß,8α-diol-9ß,10ß-epoxide B[a]P-7,8-diol-9,10-epoxide-1		
Benzo[a]pyrene, 7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-, (+)	63323-31-9	7R,8S,9S,10R
Synonyms: Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, [7R-(7.alpha.,8.beta.,8a.alpha.,9a.alpha.)]- (+)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (+)-Benzo[a]pyrene-7ß,8α-diol-9α,10α-epoxide 2 (+)-Benzo[a]pyrene-7,8-diol-9,10-epoxide 2		
Benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (-)	63323-30-8	7S,8R,9R,10S
Synonyms: Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, [7S-(7.alpha.,8.beta.,8a.alpha.,9a.alpha.)]- (-)-7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Benzo[a]pyrene-7α,8ß-diol-9ß,10ß-epoxide 2 (-) Benzo[a]pyrene-7,8-diol-9,10-epoxide 2		
Benzo[a]pyrene, 7α,8ß-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-, (±)	58917-67-2	
Synonyms: Benzo[10,11]chryseno[3,4-b]oxirene-7,8-diol, 7,8,8a,9a-tetrahydro-, (7.alpha.,8.beta.,8a.beta.,9a.beta.) (±)-trans-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (±)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Benzo[a]pyrene-7ß,8α-diol-9α,10α-epoxide Benzo[a]pyrene-7ß,8α-diol-9α,10α-epoxide Trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (anti) B[a]P-7,8-diol-9,10-epoxide 2 Benzo[a]pyrene-7,8-diol-9,10-epoxide 2		

E1.3.3 Benzo[a]pyrene 9,10-diol-7,8-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[a]pyrene, 9,10-dihydroxy-7,8-epoxy-7,8,9,10-tetrahydro-, (±)	57527-54-9	
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene-8,9-diol, 6b,7a,8,9-tetrahydro- 9,10-dihydrodiol-7,8-epoxybenzo[a]pyrene Benzo[a]pyrene-9,10-diol-7,8-epoxide Benzo[a]pyrene-9,10-dihydrodiol-7,8-oxide		
Benzo[a]pyrene, 9β,10α-dihydroxy-7α,8α-epoxy-7,8,9,10-tetrahydro-,	64937-37-7	7S,8R,9S,10R
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene-8,9-diol, 6b,7a,8,9-tetrahydro-, [6bS-(6b.alpha.,7a.alpha.,8.alpha.9.beta.)]- Trans-9β,10α-dihydroxy-7α,8α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene		

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[a]pyrene, 9α,10β-dihydroxy-7β,8β-epoxy-7,8,9,10-tetrahydro-, (±)	58116-22-6	
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene-8,9-diol, 6b,7a,8,9-tetrahydro-, (6b.alpha.,7.alpha.,8.alpha.,9.beta.)-, (.+-.)- Benzo[a]pyrene, 9β,10α-dihydroxy-7α,8α-epoxy-7,8,9,10-tetrahydro-, (±) Benzo[a]pyrene-9,10-diol-7,8-epoxide 1		
Benzo[a]pyrene, 9α,10β-dihydroxy-7α,8α-epoxy-7,8,9,10-tetrahydro-,	126642-38-4	7S,8R,9R,10S
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene-8,9-diol, 6b,7a,8,9-tetrahydro-, [6bS-(6b.alpha.,7a.alpha.,8.beta.,9.alpha.)]- Trans-9α,10β-dihydroxy-7α,8α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene		
Benzo[a]pyrene, 9β,10α-dihydroxy-7β,8β-epoxy-7,8,9,10-tetrahydro-,	64937-38-8	7R,8S,9S,10R
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene-8,9-diol, 6b,7a,8,9-tetrahydro-, [6bR-(6b.alpha.,7a.alpha.,8.beta.,9.alpha.)]- Trans-9β,10α-dihydroxy-7β,8β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene		
Benzo[a]pyrene, 9α,10β-dihydroxy-7α,8α-epoxy-7,8,9,10-tetrahydro-, (±)	68366-05-2	
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene-8,9-diol, 6b,7a,8,9-tetrahydro-, (6b.alpha.,7a.alpha.,8.beta.,9.alpha.)-, (.+-.) Benzo[a]pyrene, 9β,10α-dihydroxy-7β,8β-epoxy-7,8,9,10-tetrahydro-, (±) Benzo[a]pyrene-9,10-diol-7,8-epoxide 2		

E1.3.4 Benzo[a]pyrenes Epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[a]pyrene, 4,5-dihydro-4,5-epoxy-, (±)	64437-52-1	
Synonyms: Benzo[1,2]pyreno[4,5-b]oxirene, 3b,4a-dihydro-, (.+-.) Benzo[a]pyrene-4,5-epoxide Benzo[a]pyrene-4,5-oxide Benzo[a]pyrene-4,5-epoxide Benzo[a]pyrene-4,5-oxide		
Benzo[a]pyrene, 7,8-dihydro-7,8-epoxy-, (±)	64437-54-3	
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene, 6b,7a-dihydro-, (.+-.) Benzo[a]pyrene-7,8-epoxide Benzo[a]pyrene-7,8-oxide		
Benzo[a]pyrene, 7,8-dihydro-7,8-epoxy-, (+)-	68906-82-1	7R,8S
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene, 6b,7a-dihydro- [6bR]- (+) Benzo[a]pyrene 7,8-oxide		
Benzo[a]pyrene, 7,8-dihydro-7,8-epoxy-, (-)	68907-98-2	7S,8R
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene, 6b,7a-dihydro-[6bS]-, (.-.) (-) Benzo[a]pyrene 7,8-oxide		
Benzo[a]pyrene, 7,8-epoxy-7,8,9,10-tetrahydro-, (±)	36504-67-3	
Synonyms: Benzo[10,11]chryseno[1,2-b]oxirene, 6b,7a,8,9-tetrahydro-, 7,8-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene Benzo[a]pyrene-7,8-epoxide-7,8,9,10-tetrahydro-		
Benzo[a]pyrene, 9,10-dihydro-9,10-epoxy-, (±)	64437-53-2	
Synonyms: Benzo[10,11]chryseno[3,4-b]oxirene, 8a,9a-dihydro-, (.+-.) Benzo[a]pyrene-9,10-epoxide Benzo[a]pyrene-9,10-oxide		
Benzo[a]pyrene, 9,10-epoxy-7,8,9,10-tetrahydro-, (±)	64608-56-6	
Synonyms: Benzo[10,11]chryseno[3,4-b]oxirene, 7,8,8a,9a-tetrahydro-, (.+-.) 9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene		
Benzo[a]pyrene, 11,12-dihydro-11,12-epoxy-, (±)	67445-96-9	
Synonyms: Benzo[10,11]chryseno[5,6-b]oxirene, 1a,11c-dihydro-, (.+-.) Benzo[a]pyrene-11,12-epoxide Benzo[a]pyrene-11,12-oxide		

E1.3.5 Benzo[a]pyrene Quinones

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[a]pyrene, 1,6-quinone-,	003067-13-8	
Synonyms: Benzo[a]pyrene, 1,6-dione- Benzo[a]pyrene-1,6-quinone B[a]P-1,6-quinone	003067-13-8 003067-13-8 003067-13-8	
Benzo[a]pyrene, 3,6-quinone-,	003067-14-9	
Synonyms: Benzo[a]pyrene, 3,6-dione- Benzo[a]pyrene-3,6-quinone B[a]P-3,6-quinone	003067-14-9 003067-14-9 003067-14-9	
Benzo[a]pyrene, 6,12-quinone-,	003067-12-7	
Synonyms: Benzo[a]pyrene, 6,12-dione Benzo[a]pyrene-6,12-quinone B[a]P-6,12-quinone	003067-12-7 003067-12-7 003067-12-7	

E1.4.1 Benzo[c]phenanthrene Diols

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[c]phenanthrene, 1,2-dihydroxy-1,2-dihydro-, trans-, (±)	73093-22-8	
Synonyms: Benzo[c]phenanthrene-1,2-diol, 1,2-dihydro-, trans-, Benzo[c]phenanthrene, 1α,2ß-dihydroxy-1,2-dihydro-, (±) Benzo[c]phenanthrene, 1ß,2α-dihydroxy-1,2-dihydro-, (±) Trans-1,2-dihydroxy-1,2-dihydrobenzo[c]phenanthrene Benzo[c]phenanthrene-1,2-dihydrodiol		
Benzo[c]phenanthrene, 3,4-dihydroxy-3,4-dihydro-, trans-, (±)	79117-84-3	
Synonyms: Benzo[c]phenanthrene-3,4-diol, 3,4-dihydro-, trans-(.+-.) Benzo[c]phenanthrene, 3α,4ß-dihydroxy-3,4-dihydro-, (±) Benzo[c]phenanthrene, 3ß,4α-dihydroxy-3,4-dihydro-, (±) Trans-3,4-dihydroxy-3,4-dihydrobenzo[c]phenanthrene Benzo[c]phenanthrene-3,4-dihydrodiol		
Benzo[c]phenanthrene, 3,4-dihydroxy-1,2,3,4-tetrahydro-, trans-, (±)	86602-29-1	
Synonyms: Benzo[c]phenanthrene-3,4-diol, 1,2,3,4-tetrahydro-, trans-(.+-.) Benzo[c]phenanthrene, 3α,4ß-dihydroxy-1,2,3,4-tetrahydro-, (±) Benzo[c]phenanthrene, 3ß-4α-dihydroxy-1,2,3,4-tetrahydro-, (±) Trans-3,4-dihydroxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene Benzo[c]phenanthrene-1,2,3,4-tetrahydro-3,4-diol		
Benzo[c]phenanthrene, 5,6-dihydroxy-5,6-dihydro-, trans-, (±)	101313-28-4	
Synonyms: Benzo[c]phenanthrene-5,6-diol, 5,6-dihydro-, trans-(.+-.) Benzo[c]phenanthrene, 5α,6ß-dihydroxy-5,6-dihydro-, (±) Benzo[c]phenanthrene, 5ß-6α-dihydroxy-5,6-dihydro-, (±) Trans-5,6-dihydroxy-5,6-dihydrobenzo[c]phenanthrene Benzo[c]phenanthrene-5,6-dihydrodiol		

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[c]phenanthrene,3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (+)	82510-56-3	1S,2R,3R,4S
Synonyms: Benzo[5,6]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11d-tetrahydro-, [1aR,(1.a.alpha.2.alpha.,3.beta.,11d.alpha.)]- (+) cis Benzo[c]phenathrene-3,4-diol-1,2-epoxide 1		
Benzo[c]phenanthrene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (-)	82510-59-6	1R,2S,3S,4R
Synonyms: Benzo[5,6]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11d-tetrahydro-, [1aS-(1a.alpha.,2.alpha.,3.beta.,11d.alpha.)]- (-) cis Benzo[c]phenathrene-3,4-diol-1,2-epoxide 1		
Benzo[c]phenanthrene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±)	75443-72-0	
Synonyms: Benzo[5,6]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11d-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,11d.alpha.)-(.+-.) Benzo[c]phenanthrene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±) Benzo[c]phenanthrene-3,4-diol-1,2-epoxide 1		
Benzo[c]phenanthrene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (+)	82510-58-5	1R,2S,3R,4S
Synonyms: Benzo[5,6]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11d-tetrahydro-, [1aS-(1a.alpha.,2.beta.,3,.alpha.11d.alpha.)]- (+) trans Benzo[c]phenanthrene-3,4-diol-1,2-epoxide 2		
Benzo[c]phenenthrene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro (-)	82510-57-4	1S,2R,3S,4R
Synonyms: Benzo[5,6]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11d-tetrahydro-, [1aR-(1a.alpha.,2,beta.,3.alpha.,11d.alpha.)]- (-) trans Benzo[c]phenanthrene-3,4-diol-1,2-epoxide 2		
Benzo[c]phenanthrene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	75410-89-8	
Synonyms: Benzo[5,6]phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,11d-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,11d.alpha.)-(.+-.) Benzo[c]phenanthrene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±) Benzo[c]phenanthrene-3,4-diol-1,2-epoxide 2		

E1.4.3 Benzo[c]phenanthrene 1,2-diol-3,4-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[c]phenanthrene, 1α,2ß-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (±)	83730-23-9	
Synonyms: Benzo[5,6]phenanthr[1,2-b]oxirene-10,11-diol, 1a,10,11,11a-tetrathydro-, (1a.alpha.10.beta.,11.alpha.,11a.alpha.)- Benzo[c]phenanthrene, 1ß,2α-dihydroxy-3ß,4ß-epoxy-1,2,3,4-tetrahydro-, (±) Benzo[c]phenanthrene-1,2-diol-3,4-epoxide 1		
Benzo[c]phenanthrene, 1α,2ß-dihydroxy-3ß,4ß-epoxy-1,2,3,4-tetrahydro-, (±)	83730-36-3	
Synonyms: Benzo[5,6]phenanthr[1,2-b]oxirene-10,11-diol, 1a,10,11,11a-tetrathydro-, (1a.alpha.,10.alpha.,11.beta.,11a.alpha.)- Benzo[c]phenanthrene, 1ß,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (±) Benzo[c]phenanthrene-1,2-diol-3,4-epoxide 2		

E1.5 Benzo[e]pyrenes

E1.5.1 Benzo[e]pyrene Diols

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[e]pyrene, 4,5-dihydroxy-4,5-dihydro-, trans-, (±)	66788-09-8	
Synonyms: Benzo[e]pyrene-4,5-diol, 4,5-dihydro-, Benzo[e]pyrene, 4α,5ß-dihydroxy-4,5-dihydro-, (±) Benzo[e]pyrene, 4ß,5α-dihydroxy-4,5-dihydro-, (±) Trans-4,5-dihydroxy-4,5-dihydrobenzo[e]pyrene Benzo[e]pyrene-4,5-dihydrodiol		
Benzo[e]pyrene, 9,10-dihydroxy-9,10-dihydro-, trans-, (±)	74634-55-2	
Synonyms: Benzo[e]pyrene-9,10-diol, 9,10-dihydro-, trans-(.+-.) Benzo[e]pyrene, 9α,10ß-dihydroxy-9,10-dihydro-, (±) Benzo[e]pyrene, 9ß,10α-dihydroxy-9,10-dihydro-, (±) Trans-9,10-dihydroxy-9,10-dihydrobenzo[e]pyrene Benzo[e]pyrene-9,10-dihydrodiol		
Benzo[e]pyrene, 9,10-dihydroxy-9,10,11,12-tetrahydro-, trans-, (±)	66788-03-2	
Synonyms: Benzo[e]pyrene-9,10-diol, 9,10,11,12-tetrahydro-, trans-, Benzo[e]pyrene, 9α,10ß-dihydroxy-9,10,11,12-tetrahydro-, (±) Benzo[e]pyrene, 9ß,10α-dihydroxy-9,10,11,12-tetrahydro-, (±) Trans-9,10-dihydroxy-9,10,11,12-tetrahydrobenzo[e]pyrene		

E1.5.2 Benzo[e]pyrene 9,1-diol-11,12-epoxide

Compound/Synonyms		CAS #	Absolute Configuration
Benzo[e]pyrene, 9,10-dihydroxy-11,12-epoxy-9,10,11,12-tetrahydro-, (±)		71213-91-7	
Synonyms:	Benzo[8,9]triphenyleno[1,2-b]oxirene-9,10-diol, 9,10,10a,11a-tetrahydro-, Benzo[e]pyrene-9,10-diol-11,12-epoxide		
Benzo[e]pyrene, 9α,10ß-dihydroxy-11α,12α-epoxy-9,10,11,12-tetrahydro-, (±)		74465-36-4	
Synonyms:	Benzo[8,9]triphenyleno[1,2-b]oxirene-9,10-diol, 9,10,10a,11a-tetrahydro-, (9.alpha.,10.beta.,10a.beta.,11a.beta.)-(.+-.) Benzo[e]pyrene, 9ß,10α-dihydroxy-11ß,12ß-epoxy-9,10,11,12-tetrahydro-, (±) (±)-9ß,10α-dihydroxy-11ß,12ß-epoxy-9,10,11,12-tetrahydrobenzo[e]pyrene Benzo[e]pyrene-9,10-diol-11,12-epoxide 1		
Benzo[e]pyrene, 9α,10ß-dihydroxy-11ß,12ß-epoxy-9,10,11,12-tetrahydro-, (±)		74465-37-5	
Synonyms:	Benzo[8,9]triphenyleno[1,2-b]oxirene-9,10-diol, 9,10,10a,11a-tetrahydro-, (9.alpha.,10.beta.,10a.alpha.,11a.alpha.)-(.+-.) Benzo[e]pyrene, 9ß,10α-dihydroxy-11α,12α-epoxy-9,10,11,12-tetrahydro-, (±) (±)-9ß,10α-dihydroxy-11α,12α-epoxy-9,10,11,12-tetrahydrobenzo[e]pyrene Benzo[e]pyrene-9,10-diol-11,12-epoxide 2		

E1.5.3 Benzo[e]pyrene Epoxides

Compound/Synonyms		CAS #	Absolute Configuration
Benzo[e]pyrene, 9,10-epoxy-9,10,11,12-tetrahydro-, (±)		82132-21-6	
Synonyms:	Benzo[8,9]triphenyleno[1,2-b]oxirene, 9,10,10a,11a-tetrahydro-, (.+-.) Benzo[e]pyrene, 9,10,11,12-tetrahydro-9,10epoxide 9,10-epoxy-9,10,11,12-tetrahydrobenzo[e]pyrene		

E1.6 Benzo[rst]pentaphene (Dibenzo[a,l]pyrene)

E1.6.1 Benzo[rst]pentaphene Diol-Epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Benzo[rst]pentaphene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (+)	73691-55-1	1S,2R,3S,4R
Synonyms: Benzo[13,14]pentapheno[1,2-b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,13c.alpha.)-(+)- (+) trans-3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrobenzo[rst]pentaphene Dibenzo[a,i]pyrene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (+) (+) trans-3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrodibenzo[a,i]pyrene		
Benzo[rst]pentaphene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±)	85718-90-7	
Synonyms: Benzo[13,14]pentapheno[1,2-b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,13c.alpha.)- Benzo[rst]pentaphene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±) (±) trans-3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrobenzo[rst]pentaphene Dibenzo[a,i]pyrene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±) trans-3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrodibenzo[a,i]pyrene		
Benzo[rst]pentaphene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	78919-12-7	
Synonyms: Benzo[13,14]pentapheno[1,2-b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,13c.alpha.)-(.+-.)- Benzo[rst]pentaphene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±) (±) trans-3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[rst]pentaphene Dibenzo[a,i]pyrene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±) trans-3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrodibenzo[a,i]pyrene		

E1.7 Cholanthrenes

Compound/Synonyms	CAS #	Absolute Configuration
Cholanthrene, 3-methyl-,	56-49-5	
Synonyms: Benz[j]aceanthrylene-1,2-dihydro, 3-methyl-, 20-methylcholanthrene 3-methylcholanthrene		
Cholanthrene, 2a,3-dihydroxy-3-methyl-, cis-, (±)	68688-84-6	
Synonyms: Benz[j]aceanthrylene-2a, 3(3H)-diol, 1,2-dihydro-3-methyl-, Cis-2a,3-dihydroxy-3-methylcholanthrene		

E1.7.1 Cholanthrene Diols

Compound/Synonyms	CAS #	Absolute Configuration
Cholanthrene, 4,5-dihydroxy-4,5-dihydro-3-methyl-, trans-, (±)	68688-85-7	
Synonyms: Benz[j]aceanthrylene-4,5-diol, 1,2,4,5-tetrahydro-3-methyl-, Cholanthrene, 4α,5ß-dihydroxy-3-methyl-, (±) Cholanthrene, 4ß,5α-dihydroxy-4,5-dihydro-3-methyl-, (±) Trans-4,5-dihydroxy-4,5-dihydro-3-methylcholanthrene		
Cholanthrene, 7,8-dihydroxy-7,8-dihydro-3-methyl-,trans-, (±)	68688-86-8	
Synonyms: Benz[j]aceanthrylene-7,8-diol, 1,2,7,8-tetrahydro-3-methyl-, trans-, Cholanthrene, 7α,8ß-dihydroxy-7,8-dihydro-3-methyl-, (±) Cholanthrene, 7ß,8α-dihydroxy-7,8-dihydro-3-methyl-, (±) Trans-7,8-dihydroxy-7,8-dihydro-3-methylcholanthrene		
Cholanthrene, 9,10-dihydroxy-9,10-dihydro-3-methyl-, trans-, (±)	68688-87-9	
Synonyms: Benz[j]aceanthrylene-9,10-diol, 1,2,9,10-tetrahydro-3-methyl-, trans-, Cholanthrene, 9α,10ß-dihydroxy-9,10-dihydro-3-methyl-, (±) Cholanthrene, 9ß,10α-dihydroxy-9,10-dihydro-3-methyl-, (±) Trans-9,10-dihydroxy-9,10-dihydro-3-methylcholanthrene Trans-9,10-dihydro-9,10-dihydroxy-3-methylcholanthrene		
Cholanthrene, 11,12-dihydroxy-11,12-dihydro-3-methyl-, trans-, (±)	105967-38-2	
Synonyms: Benz[j]aceanthrylene-11,12-diol, 1,2,11,12-tetrahydro-3-methyl-, trans-, Cholanthrene, 11α,12ß-dihydroxy-11,12-dihydro-3-methyl-, (±) Cholanthrene, 11ß,12α-dihydroxy-11,12-dihydro-3-methyl-, (±) Trans-11,12-dihydroxy-11,12-dihydro-3-methylcholanthrene Trans-11,12-dihydro-11,12-dihydroxy-3-methylcholanthrene		

E1.8 Chrysene

E1.8.1 Chrysene Diols

Compound/Synonyms	CAS #	Absolute Configuration
Chrysene, 1α,2ß-dihydroxy-1,2-dihydro-, (+)	80433-95-0	1S,2S
Synonyms: 1,2-chrysenediol, 1,2-dihydro-, (1S-trans)- (+)-(1S,2S)-1α,2ß-dihydroxy-1,2-dihydrochrysene (+)-chrysene-1,2-dihydrodiol		
Chrysene, 1ß,2α-dihydroxy-1,2-dihydro-, (-)	77123-18-3	1R,2R
Synonyms: 1,2-chrysenediol, 1,2-dihydro-, (1R-trans)- (-)-(1R,2R)-1ß,2α-dihydroxy-1,2-dihydrochrysene (-)-chrysene-1,2-dihydrodiol		
Chrysene, 1,2-dihydroxy-1,2-dihydro-, trans-, (±)	67175-76-2	
Synonyms: 1,2-chrysenediol, 1,2-dihydro-, trans-(.+-.) Chrysene, 1α,2ß-dihydroxy-1,2-dihydro-, (±) Chrysene, 1ß,2α-dihydroxy-1,2-dihydro-, (±) Trans-1,2-dihydroxy-1,2-dihydrochrysene (±)-chrysene-1,2-dihydrodiol Chrysene-1,2-dihydrodiol		
Chrysene, 3,4-dihydroxy-3,4-dihydro-, trans-, (±)	67175-77-3	
Synonyms: 3,4-chrysenediol, 3,4-dihydro-, trans-(.+-.) Chrysene, 3α,4ß-dihydroxy-3,4-dihydro-, (±) Chrysene, 3ß,4α-dihydroxy-3,4-dihydro-, (±) Trans-3,4-dihydroxy-3,4-dihydrochrysene (±)-chrysene-3,4-dihydrodiol Chrysene-3,4-dihydrodiol		
Chrysene, 5,6-dihydroxy-5,6-dihydro-, trans-, (±)	67175-78-4	
Synonyms: 5,6-chrysenediol, 5,6-dihydro-, trans-(.+-.) Chrysene, 5α,6ß-dihydroxy-5,6-dihydro-, (±) Chrysene, 5ß,6α-dihydroxy-5,6-dihydro-, (±) Trans-5,6-dihydroxy-5,6-dihydrochrysene (±)-chrysene-5,6-dihydrodiol Chrysene-5,6-dihydrodiol		

E1.8.2 Chrysene Epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Chrysene, 1,2-epoxy-1,2,3,4-tetrahydro-, (±)	72045-57-9	
Synonyms: Chryseno[1,2-b]oxirene, 1a,2,3,11b-tetrahydro-, 1,2-epoxy-1,2,3,4-tetrahydrochrysene Chrysene-H4-1,2-epoxide		
Chrysene, 3,4-epoxy-1,2,3,4-tetrahydro-, (±)	67694-88-6	
Synonyms: Chryseno[3,4-b]oxirene, 1,2,2a,3a-tetrahydro-, 3,4-epoxy-1,2,3,4-tetrahydrochrysene Chrysene-H4-3,4-epoxide		

E1.8.3 Chrysene 3,4-diol-1,2-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Chrysene, 3B,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-,	77255-40-4	1S,2R,3S,4R
Synonyms: Chryseno[1,2-b]oxirene-2,3-diol,1a,2,3,11b-tetrahydro-, [1aR-(1a.alpha.,2.alpha.,3.beta.,11b.alpha.)]- Trans-3B,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrochrysene		
Chrysene, 3α,4B-dihydroxy-1B,2B-epoxy-1,2,3,4-tetrahydro-, (±)	83830-70-0	
Synonyms: Chryseno[1,2-b]oxirene-2,3-diol,1a,2,3,11b-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,11b.alpha.)- Chrysene, 3B,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±) Trans-3,4-dihydroxy-syn-1,2-epoxy-1,2,3,4-tetrahydrochrysene		
Chrysene, 3α,4B-dihydroxy-1B,2B-epoxy-1,2,3,4-tetrahydro-,	77255-44-8	1R,2S,3S,4R
Synonyms: Chryseno[1,2-b]oxirene-2,3-diol,1a,2,3,11b-tetrahydro-, [1aS-(1a.alpha.,2.beta.,3.alpha.,11b.alpha.)]- Trans-3α,4B-dihydroxy-1B,2B-epoxy-1,2,3,4-tetrahydrochrysene		
Chrysene, 3α,4B-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	70951-83-6	
Synonyms: Chryseno[1,2-b]oxirene-2,3-diol,1a,2,3,11b-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,11b.alpha.)- Chrysene, 3B,4α-dihydroxy-1B,2B-epoxy-1,2,3,4-tetrahydro-, (±) Trans-3,4-dihydroxy-anti-1,2-epoxy-1,2,3,4-tetrahydrochrysene		

E1.8.4 Chrysene 1,2-diol-3,4-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Chrysene, 1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (+)	80433-80-3	1S,2R,3S,4R
Synonyms: Chryseno[3,4-b]oxirene-1,2-diol, 1,2,2a,3a-tetrahydro-, [1S-(1.alpha.,2.beta.,2a.beta.,3a.beta.)]- (+)-(1S,2R,3S,4R)-1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrochrysene (+)-chrysene-1,2-diol-3,4-epoxide 1		
Chrysene, 1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (-)	77123-24-1	1R,2S,3R,4S
Synonyms: Chryseno[3,4-b]oxirene-1,2-diol, 1,2,2a,3a-tetrahydro-, [1R-(1.alpha.,2.beta.,2a.beta.,3a.beta.)]- (-)-(1R,2S,3R,4S)-1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydrochrysene (-) Chrysene1,2-diol-3,4-epoxide 1		
Chrysene, 1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (±)	72074-67-0	
Synonyms: Chryseno[3,4-b]oxirene-1,2-diol, 1,2,2a,3a-tetrahydro-, (.+-.) Chrysene, 1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (±) (±)-1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydrochrysene Chrysene-1,2-diol-3,4-epoxide 1		
Chrysene, 1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (+)	77123-23-0	1R,2S,3S,4R
Synonyms: Chryseno[3,4-b]oxirene-1,2-diol, 1,2,2a,3a-tetrahydro-, [1R-(1.alpha.,2.beta.,2a.alpha.,3a.alpha.)]- (+)-(1R,2S,3S,4R)-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrochrysene (+)-chrysene-1,2-diol-3,4-epoxide 2		
Chrysene, 1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (-)	80433-82-5	1S,2R,3R,4S
Synonyms: Chryseno[3,4-b]oxirene-1,2-diol, 1,2,2a,3a-tetrahydro-, [1S-(1.alpha.,2.beta.,2a.alpha.,3a.alpha.)]- (-)-(1S,2R,3R,4S)-1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydrochrysene (-)-chrysene-1,2-diol-3,4-epoxide 2		
Chrysene, 1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (±)	72074-69-9	
Synonyms: Chryseno[3,4-b]oxirene-1,2-diol, 1,2,2a,3a-tetrahydro-, (1.alpha.,2.beta.,2a.beta.,3a.beta.)-(.+-.) Chrysene, 1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (±) (±)-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrochrysene (±)-chrysene-1,2-diol-3,4-epoxide 2 Chrysene-1,2-diol-3,4-epoxide 2		

E1.9.1 Dibenz[a,j]anthracene Diol-Epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Dibenz[a,j]anthracene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-,	121209-31-2	1S,2R,3S,4R
Synonyms: Naphtho[1',2':6,7]phenanthro[3,4b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, [1aS-(1a.alpha.,2.alpha.,3.beta.,13c.alpha.)]-Trans-3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrodibenz[a,j]anthracene,		
Dibenz[a,j]anthracene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-,	121209-32-3	1R,2S,3R,4S
Synonyms: Naphtho[1',2':6,7]phenanthro[3,4b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, [1aR-(1a.alpha.,2.alpha.,3.beta.,13c.alpha.)]-Trans-3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrodibenz[a,j]anthracene,		
Dibenz[a,j]anthracene, 3ß,4α-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	131486-57-2	
Synonyms: Naphtho[1',2':6,7]phenanthro[3,4b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, (1a.alpha.,2,alpha.,3.beta.,13c.alpha.)-(.+-.)-Dibenz[a,j]anthracene, 3α,4ß-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±)		
Dibenz[a,j]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-,	121209-33-4	1S,2R,3R,4S
Synonyms: Naphtho[1',2':6,7]phenanthro[3,4b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, [1aR-(1a.alpha.,2.beta.,3.alpha.,13c.alpha.)]-Trans-3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrodibenz[a,j]anthracene,		
Dibenz[a,j]anthracene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-,	121209-34-5	1R,2S,3S,4R
Synonyms: Naphtho[1',2':6,7]phenanthro[3,4b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, [1aS-(1a.alpha.,2.beta.,3.alpha.,13c.alpha.)]-Trans-3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydrodibenz[a,j]anthracene,		
Dibenz[a,j]anthracene, 3α,4ß-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydro-, (±)	119181-08-7	
Synonyms: Naphtho[1',2':6,7]phenanthro[3,4b]oxirene-2,3-diol, 1a,2,3,13c-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,13c.alpha.)-(.+-.)-Dibenz[a,j]anthracene, 3ß,4α-dihydroxy-1ß,2ß-epoxy-1,2,3,4-tetrahydro-, (±)		

E1.10 Phenanthrenes

E1.10.1 Phenanthrene Diols

Compound/Synonyms	CAS #	Absolute Configuration
Phenanthrene, 1,2-dihydroxy-1,2-dihydro-, trans-, (±)	103302-39-2	
Synonyms: 1,2-phenanthrenediol, 1,2-dihydro-, trans-(.+-.) Phenanthrene, 1α,2ß-dihydroxy-1,2-dihydro-, (±) Phenanthrene, 1ß,2α-dihydroxy-1,2-dihydro-, (±) Trans-1,2-dihydroxy-1,2-dihydrophenanthrene Phenanthrene-1,2-dihydrodiol		
Phenanthrene, 3,4-dihydroxy-3,4-dihydro (±)	569-20-0	
Synonyms: 3,4-phenanthrenediol,3,4-dihydro-,trans- Trans-3,4-dihydroxy-3,4-dihydrophenanthrene Phenanthrene,3,4-dihydrodiol		
Phenanthrene, 9,10-dihydroxy-9,10-dihydro-, trans-, (±)	25061-61-4	
Synonyms: 9,10-phenanthrendiol, 9,10-dihydro-, trans-(.+-.)- Phenanthrene, 9α,10ß-dihydroxy-9,10-dihydro-, (±) Trans-9,10-dihydroxy-9,10-dihydrophenanthrene Phenanthrene, 9ß,10α-dihydroxy-9,10-dihydro-, (±) Phenanthrene-9,10-dihydrodiol		

E1.10.2 Phenanthrene Epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Phenanthrene, 1,2-epoxy-1,2,3,4-tetrahydro-, (±)	56179-80-7	
Synonyms: Phenanthro[1,2-b]oxirene, 1a,2,3,9b-tetrahydro-, 1,2-epoxy-1,2,3,4-tetrahydrophenanthrene,		
Phenanthrene, 3,4-epoxy-1,2,3,4-tetrahydro-, (±)	66226-26-4	
Synonyms: Phenanthro[3,4-b]oxirene, 1a,2,3,9c-tetrahydro-, 3,4-epoxy-1,2,3,4-tetrahydrophenanthrene,		

E1.10.3 Phenanthrene 1,2-diol-3,4-epoxides

Compound/Synonyms	CAS #	Absolute Configuration
Phenanthrene, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-, (±)	75947-56-7	
Synonyms: Phenanthro [3,4-b] oxirene-2,3-diol, 1a,2,3,9c-tetrahydro-, 1,2-diol-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene Phenanthrene-1,2-dihydrodiol-3,4-epoxide Phenanthrene-1,2-diol-3,4-epoxide Phenanthrene-1,2-diol-3,4-oxide		
Phenanthrene, 1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-,	77123-21-8	1S,2R,3S,4R
Synonyms: Phenanthro [3,4-b] oxirene-2,3-diol, 1a,2,3,9c-tetrahydro-, [1aS-(1a.alpha.,2.alpha.,3.beta.,9c.alpha.)]- Trans-1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrophenanthrene		
Phenanthrene, 1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (±)	72074-69-2	
Synonyms: Phenanthro [3,4-b] oxirene-2,3-diol, 1a,2,3,9c-tetrahydro-, (1a.alpha.,2.alpha.,3.beta.,9c.alpha.)-,(.+-.)- Phenanthrene,1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro (±) (±)-1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydrophenanthrene Phenanthrene 1,2-diol-3,4-epoxide 1		
Phenanthrene, 1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-,	77123-22-9	1S,2R,3R,4S
Synonyms: Phenanthro [3,4-b] oxirene-2,3-diol, 1a,2,3,9c-tetrahydro-, [1aR-(1a.alpha.,2.beta.,3.alpha.,9c.alpha.)]- Trans-1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydrophenanthrene		
Phenanthrene, 1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro (±)	72074-68-1	
Synonyms: Phenanthro[3,4-b]oxirene-2,3-diol, 1a,2,3,9c-tetrahydro-, (1a.alpha.,2.beta.,3.alpha.,9c.alpha.) Phenanthrene, 1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (±) (±)-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrophenanthrene Phenanthrene,1,2-diol-3,4-epoxide 2		

E1.11 Triphenylene

E1.11.1 Triphenylene Diol-Epoxides

Compound/Synonyms		CAS #	Absolute Configuration
Triphenylene, 1α,2β-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-,(±)		74465-39-7	
Synonyms:	Triphenyleno[1,2-b]oxirene-9,10-diol, 9,10,10a,11a-tetrahydro-, (9.alpha.,10.beta.,10a.alpha.,11a.alpha.)-(.+-.)- Triphenylene, 1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-,(±) (±)-trans-1α,2β-dihydroxy-3β-4β-epoxy-1,2,3,4-tetrahydrotriphenylene		
Triphenylene, 1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydro-, (±)		74465-38-6	
Synonyms:	Triphenyleno[1,2-b]oxirene-9,10-diol, 9,10,10a,11a-tetrahydro-, (9.alpha.,10.beta.,10a.beta.,11a.beta.)-(.+-.)- Triphenylene, 1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydro-, (±) (±)-trans- 1α,2β-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrotriphenylene		